Genetic Risk Assessment in Myeloproliferative Neoplasms

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CME Activity

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Abstract

The World Health Organization classification system recognizes 4 variants of *JAK2* mutation–enriched myeloproliferative neoplasms (for expansion of gene symbols, use search tool at www.genenames.org): essential thrombocythemia (ET), polycythemia vera (PV), primary myelofibrosis (PMF), and prefibrotic PMF. All 4 disorders are characterized by stem cell–derived clonal myeloproliferation with mutually exclusive driver mutations, including *JAK2*, *CALR*, and *MPL*. The median survival is approximately 20 years for ET, 14 years for PV, and 6 years for PMF; age is the most important determinant of survival with the corresponding median of 33, 24, and 15 years in patients younger than 60 years. Genetic information is the second most important prognostic tool and includes karyotype, driver mutational status, and presence of specific other mutations. Karyotype has been shown to carry prognostic relevance in PV (abnormal vs normal) and PMF (unfavorable vs favorable abnormalities). Driver mutational status is prognostically most relevant in PMF; type 1/type 1-like *CALR* vs other driver mutational status has been associated with superior survival. In ET, arterial thrombosis risk is higher in patients with *JAK2* or *MPL* mutations whereas *MPL*-mutated patients might be at risk for accelerated fibrotic progression. *ASXL1* and *SRSF2* mutations have been associated with inferior overall, leukemia-free, or fibrosis-free survival in both PV and PMF, and a recent targeted sequencing study has identified additional other adverse mutations in

both these disorders, as well as in ET. Further enhancement of genetic risk stratification in myeloproliferative neoplasms is possible by combining cytogenetic and mutation information and developing a prognostic model that is adjusted for age.

 ■ Mayo Clin Proc. 2017;92(8):1283-1290

Myeloproliferative neoplasms (MPN) constitute one of several categories of myeloid neoplasms according to the World Health Organization (WHO) classification system for hematopoietic tumors.[1,2] The 2016 WHO MPN category includes chronic myeloid leukemia, which is invariably associated with the *BCR-ABL1* mutation (for expansion of gene symbols, use search tool at www.genenames.org); the *JAK2* mutation-enriched MPN, which include polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), and prefibrotic PMF; and other less frequent clinic-pathologic entities including chronic neutrophilic leukemia, chronic eosinophilic leukemia—not otherwise specified, and MPN—unclassifiable. The *JAK2* mutation—enriched MPN are characterized by stem cell—derived clonal myeloproliferation with mutually exclusive *JAK2*, *CALR*, and *MPL* mutations.[3,4]

Polycythemia vera is almost always associated with a *JAK2* mutation, primarily the *JAK2*V617F mutation. *JAK2*V617F is also the most frequent mutation in ET and PMF, with an incidence of 50% to 70% in both. About 40% of the patients with either ET or PMF harbor *CALR* (20%-25%), *MPL* (3%-8%), or none of the 3 driver mutations (ie, are triple negative). Accordingly, the presence, absence, or specific type of driver mutations cannot be used for diagnostic distinction among the different MPN, which is based primarily on bone marrow morphology and peripheral blood counts.[2]

PHENOTYPE

Phenotypically, PV is defined by clonal erythrocytosis, ET by clonal thrombocytosis, and PMF by characteristic bone marrow morphology. In addition, all 3 disorders might be associated with hepatosplenomegaly (PMF>PV>ET), leukocytosis (PMF>PV>ET), thrombocytosis (ET>PMF>PV), microvascular symptoms (PV>ET>PMF), constitutional symptoms (PMF>PV>ET), thrombohemorrhagic complications (PV>ET>PMF), and variable risk of leukemic transformation (PMF>PV>ET), or fibrotic progression (PV>ET).[5] Patients with PV or PMF might also experience intractable pruritus, whereas increased rates of first trimester miscarriage have been reported in ET[6,7]; in a recent study, approximately 59% of 292 patients with WHO-defined ET were women and 58% of the study population was younger than 60 years.[8] Additional clinical manifestations in PV include symptoms of hyperviscosity and in PMF, progressive anemia, leukoerythroblastosis, extramedullary hematopoiesis, recurrent splenic infarcts, peripheral edema, early satiety, cachexia, and symptoms of portal hypertension, including ascites and variceal bleeding.

Driver mutations might also influence MPN phenotype.[4] For example, *JAK2*V617F-mutated patients with ET or PMF are usually older and display higher hemoglobin and leukocyte counts and lower platelet count.[9] *JAK2* exon 12—mutated patients with PV are younger and often display predominantly erythroid myeloproliferation. In contrast, *CALR*-mutated or triple-negative patients with ET are younger and display male preponderance, higher platelet count, and lower hemoglobin and leukocyte counts. *CALR*-mutated patients with PMF are also younger and present with higher platelet count and lower frequencies of anemia and leukocytosis. The risk of arterial thrombosis in ET is significantly higher in *JAK2*- or *MPL*-mutated patients than in *CALR*-mutated or triple-negative patients; the same might be true in terms of vascular risk in PMF.[10] In regard to phenotypic correlates of mutations other than *JAK2*, *CALR*, or *MPL*, the most notable so far has been the impressively significant correlation between *U2AF1* mutations and anemia in patients with PMF.[11,12]

CLINICAL COURSE

Life expectancy of patients with MPN is worse than that of the sex- and age-matched control population, and the median survival is estimated at 20 years for ET, 14 years for PV, and 6 years for PMF[8]; the corresponding

values for patients younger than 60 years were 33, 24, and 15 years. Causes of death include leukemic transformation, with a 15-year estimate of approximately 2.1% to 5.3% for ET, 5.5% to 18.7% for PV, and more than 20% for PMF.[13] Fibrotic progression rates in ET and PV, during a similar time interval, are estimated at 4% to 11% and 6% to 14%, respectively.[13] Clinical course in patients with ET or PV might be interrupted by thrombotic and bleeding events in a substantial fraction of the patients, and the particular risk depends primarily on the presence or absence of thrombosis history. In PMF, clinical course is characterized by progressive anemia, symptomatic hepatosplenomegaly, profound constitutional symptoms, and cachexia.

MUTATIONS

JAK2 (9p24) mutations are the most prevalent mutations in PV, ET, and PMF, with respective incidences of approximately 99%, 55%, and 65%.[3,9,14] *CALR* (19p13.2) mutations are the second most prevalent driver mutations in MPN and are found primarily in ET and PMF, with approximate incidences of 15% to 30%. *MPL* (1p34) mutations are the least frequent driver mutations in MPN, with approximate incidences of 4% in ET and 8% in PMF. A recent targeted sequencing study revealed that mutations or DNA variants other than *JAK2*, *CALR*, or *MPL* are found in 81% of patients with PMF, 53% with PV, and 53% with ET.[15,16] The most frequent such mutations in PMF were *ASXL1* (36%), *TET2* (18%), *SRSF2* (18%), and *U2AF1* (16%), and 35%, 26%, 10%, and 9% of the patients harbored 1, 2, 3, or 4 or more such variants or mutations, respectively. In PV, the most frequent mutations were *TET2* (22%), *ASXL1* (12%), and *SH2B3* (9%); and in ET, *TET2* (16%), *ASXL1* (11%), *DNMT3A* (6%), and *SF3B1* (5%); the respective percentages of patients with 1, 2, or 3 or more sequence variants or mutations were 30%, 20%, and 3% for PV and 41%, 8%, and 4% for ET.

CYTOGENETIC ABNORMALITIES

Among the *JAK2* mutation–enriched MPN, an abnormal karyotype is most often seen in PMF and least often in ET. In a recent study of 826 informative patients with PMF,[17] approximately 43% displayed an abnormal karyotype at the time of their referral; among the group with an abnormal karyotype, 68% consisted of sole aberrations and 14% complex karyotype. The most frequent cytogenetic abnormalities in PMF (and their approximate incidences) were 20q- (23%), 13q- (18%), +8 (11%), +9 (10%), chromosome 1q+ (10%), and −7/7q- (7%). In PV, an abnormal karyotype is seen in approximately 15% of the patients, at the time of their diagnosis, and mostly consists of sole abnormalities. The most frequent cytogenetic abnormalities in PV include −Y, +8, +9, del(20q), and chromosome 1q+.[18] The incidence of an abnormal karyotype is approximately 7% in ET, with +9, chromosome 1q+, and +8 identified as the most frequent abnormalities.[19]

GENETIC RISK ASSESSMENT IN ET

Currently established clinical risk factors for survival in ET include advanced age, leukocytosis, and thrombosis.[20,21] In routine clinical practice, some patients with prefibrotic PMF are inaccurately diagnosed and managed as ET. A large international study highlighted the prognostic relevance of distinguishing prefibrotic PMF from true WHO-defined ET, in terms of overall, leukemia-free, and myelofibrosis-free survival, and also identified advanced age, thrombosis history, leukocytosis, and anemia as independent risk factors for overall survival; thrombosis history and extreme thrombocytosis for leukemia-free survival; and advanced age and anemia for fibrotic progression.[22] These findings were confirmed in a subsequent report limited to WHO-defined true ET,[21] in which age 60 years and above, leukocyte count 11×10^9/L or greater, and history of thrombosis were identified as independent risk factors for survival and were also mostly in line with a previous Mayo Clinic study of 605 patients with ET, in which age 60 years and above, leukocyte count 15×10^9/L or greater, history of thrombosis, anemia below the lower limits of normal, tobacco use, and diabetes were identified as independent risk factors for overall survival and extreme thrombocytosis and anemia below the lower limits of normal for leukemia-free survival.[23]

To date, neither overall nor leukemia-free survival has been correlated with karyotype in ET.[19] Whether this will change in the

future, pending larger studies with an adequate number of informative cases, remains to be seen. As for driver mutational status, prognostic relevance has been found for thrombosis-free (worse in *JAK2/MPL*-mutated cases) and myelofibrosis-free (worse in *MPL*-mutated cases) survival but not for overall or leukemia-free survival.[24-26] Interestingly, the presence of *JAK2*V617F in ET has been associated with a lower risk of fibrotic progression.[22] As mentioned above, a recent targeted sequencing study revealed that 53% of patients with ET carried sequence variants or mutations other than *JAK2/CALR/MPL*.[15] The particular study identified *SH2B3*, *SF3B1*, *U2AF1*, *TP53*, *IDH2*, and *EZH2* mutations, at least 1 of which was seen in approximately 15% of the patients, as risk factors for overall, myelofibrosis-free, or leukemia-free survival; the median survival of patients with and without adverse mutations was 9 and 22 years, respectively. Furthermore, the effect on survival of these adverse mutations was not accounted for by current clinically devised prognostic models and the observations were validated in an external cohort of patients. In the particular study, the number of mutations did not provide additional prognostic information.

Current treatment in ET is directed at prevention of thrombosis and bleeding.[27-29] In a large international study of WHO-defined ET, 10% to 20% of patients experienced thrombotic complications; thrombosis history, age above 60 years, presence of *JAK2*V617F, leukocytosis, and cardiovascular risk factors were identified as independent risk factors for arterial thrombosis,[30] whereas male sex predicted venous thrombosis. In contrast, a lower risk of thrombosis has been shown in patients with extreme thrombocytosis[30] and in those with *CALR* mutations.[31]

Mutational landscape and prognostic relevance were recently studied in 359 patients with post-PV or post-ET myelofibrosis.[32] The findings from the particular study included higher *JAK2* or *CALR* mutant allele burden in transformed cases, which did not, however, affect overall survival, and documentation of an adverse survival effect of triple-negative mutational status or *SRSF2* mutations on survival of patients with post-ET myelofibrosis. In another similar study,[33] the adverse effect of triple-negative status, in terms of blast transformation risk, and type 2/type 2-like *CALR* variants on overall survival was again found.

GENETIC RISK ASSESSMENT IN PV

In the largest international study of more than 1500 patients with PV, independent risk factors for overall survival were advanced age, leukocytosis, venous thrombosis, and abnormal karyotype and for leukemia-free survival advanced age, leukocytosis, and abnormal karyotype.[20] The prognostic relevance of karyotype in PV was recently confirmed by a Mayo Clinic study in which univariate analysis identified an abnormal karyotype as adversely affecting overall, leukemia-free, and myelofibrosis-free but not thrombosis-free survival; inferior outcome, as compared with normal karyotype, was also found for unfavorable karyotype, +9, −Y, +8, and del(20q).[34] The detrimental effect of an abnormal karyotype was independent of other risk factors, including adverse mutations.

As reiterated before, practically all patients with PV harbor a *JAK2* mutation whereas the occurrence of *CALR* or *MPL* mutations is rare.[35,36] Approximately 97% of patients with PV carry the *JAK2* exon 14 mutation (ie, *JAK2*V617F), whereas the remaining 3% carry *JAK2* mutations in other exons, including exon 12. Clinical outcome comparisons between *JAK2*V617F- and *JAK2* exon 12–mutated patients have so far not shown significant differences.[37] In contrast, a higher *JAK2*V617F allele burden has been shown to cluster with fibrotic progression in PV, but it is not clear whether the particular phenomenon represented concordant time-dependent events.[38] Most recently, a collaborative work between Mayo Clinic and the University of Florence, Italy, found a high prevalence (53%) of mutations or DNA variants, involving 27 genes other than *JAK2*, *CALR*, or *MPL* in patients with PV[15]; the most frequent mutations included *TET2* (22%), *ASXL1* (12%), and *SH2B3* (9%), and the study identified *ASXL1*, *SRSF2*, and *IDH2* mutations as "adverse," affecting overall, leukemia-free, or myelofibrosis-free survival. The prevalence of adverse mutations was 15%, and their prognostic effect was independent of conventional prognostic models; the median survival of

Mayo Clin Proc. ■ August 2017;92(8):1283-1290 ■ http://dx.doi.org/10.1016/j.mayocp.2017.06.002
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patients with and without adverse mutations was 7.7 and 16.9 years, respectively.

GENETIC RISK ASSESSMENT IN PMF

Current prognostic models in PMF are mostly based on clinical parameters and include the International Prognostic Scoring System (IPSS),[39] applicable at the time of diagnosis, and the Dynamic IPSS (DIPSS),[40] applicable at any time in the clinical course of the disease. Both IPSS and DIPSS use 5 clinical parameters with independent prognostic contribution: age above 65 years, hemoglobin count less than 10 g/dL, leukocyte count greater than 25×10^9/L, circulating blasts 1% or more, and presence of constitutional symptoms. Accordingly, 4 risk categories, with median survival ranging from 1.5 years to "not reached," have been established on the basis of the number of these risk factors.

Dynamic IPSS was subsequently revised to DIPSS plus, by including red cell transfusion need, platelet count less than 100×10^9/L, and "unfavorable" karyotype as additional independent risk factors.[41] For the purposes of DIPSS plus, unfavorable karyotype included complex karyotype or sole or 2 abnormalities that included +8, −7/7q-, i(17q), inv(3), −5/5q-, 12p-, or 11q23 rearrangement.[42,43] Dynamic IPSS plus also has 4 risk categories based on the aforementioned 8 risk factors: low (no risk factors), intermediate 1 (1 risk factor), intermediate 2 (2 or 3 risk factors), and high (≥4 risk factors), with median survival of 15.4, 6.5, 2.9, and 1.3 years, respectively.[41]

The prognostic importance of karyotype in PMF was further highlighted by exhibiting the extremely poor prognosis associated with a monosomal karyotype or inv(3)/i(17q) abnormalities[44] and subsequently by a revised cytogenetic model with 4 cytogenetic risk categories: low (normal karyotype or sole abnormalities of +9, del(13q), or loss of Y chromosome); intermediate 1 (sole abnormalities of del(20q), chromosome 1q duplication, extra sex chromosome, sole translocation); intermediate 2 (sole +8 or other autosomal trisomies, sole 5q-, other sole abnormalities not included in the low or intermediate 1 risk categories, non-monosomal complex karyotype, and ≥2 non—high-risk abnormalities); and high (monosomal karyotype or any set of abnormalities that include inv(3)/i(17q), −7/7q-, 12p-, 11q23)[45]; the respective median survivals were 4.6, 3.8, 2.6, and 0.9 years.

Several other DIPSS plus—independent risk factors in PMF have since been reported and included driver mutational status,[46-52] presence or absence of mutations other than *JAK2*, *CALR*, or *MPL*,[32,33,47,50,52-54] such as *IDH1/2*,[55] *EZH2*,[53] *SRSF2*,[56] or *ASXL1*,[52] nullizygosity for *JAK2* 46/1 haplotype,[57] low *JAK2*V617F allele burden,[58,59] and increased serum interleukin 8, interleukin 2 receptor, or serum-free light chain levels.[60,61] In one of the first studies that examined the prognostic relevance of driver and other mutations,[47] we reported the longest survival in *CALR*-mutated patients without *ASXL1* mutations ($CALR^+$ $ASXL1^-$; median survival, 10.4 years), compared with patients with other mutational combinations, including *ASXL1*-mutated cases without *CALR* mutations ($CALR^-ASXL1^+$; median survival, 2.3 years). Subsequent studies have clarified the prognostic effect of *CALR* mutations by distinguishing type 1/type 1-like from type 2/type 2-like *CALR* variants and by attributing the favorable prognostic effect to type 1/type 1-like *CALR* variants and showing no prognostic difference between type 2/type 2-like *CALR*, *JAK2*, *MPL* and triple-negative mutational status, although the latter might be associated with a higher risk of leukemic transformation.[46,49]

Most recently, targeted deep sequencing studies have identified additional adverse mutations in PMF, including *CBL*, *KIT*, *RUNX1*, *SH2B3*, and *CEBPA*.[16] The presence of any one of these mutations, or *ASXL1* or *SRSF2* mutations, was shown to have an independent adverse effect on overall (median, 3.6 years vs 8.5 years) or leukemia-free (7-year risk of leukemic transformation estimated at 25% vs 4%) survival.[16] The particular study also found the prognostic relevance of the number of adverse mutations, which was in line with an earlier observation[50]; the median survival was 8.5 years in patients with no adverse mutation vs 0.7 years in patients with 3 or more adverse mutations.

CONCLUSION

Drug therapy is currently inadequate to cure their disease or prolong survival of patients with MPN. Allogeneic stem cell transplantation (ASCT) offers the possibility of

either cure or durable disease-free remission. Unfortunately, ASCT is associated with a nontrivial risk of treatment-related death or comorbidity, such as graft versus host disease (GVHD) and chronic immunosuppression from drugs used to prevent graft rejection or alleviate GVHD. Because of their relatively longer survival and more indolent clinical course, ASCT is usually not recommended for patients with PV or ET. In PMF and post-ET or post-PV myelofibrosis, ASCT is considered if its associated risk of death or comorbidity can be justified. In other words, it is reasonable to consider ASCT in otherwise transplant-eligible patients whose risk of premature death or leukemic transformation is unacceptably high for the individual patient, primarily considering their age. Such therapeutic decision making requires a comprehensive assessment of clinical and genetic risk factors, which are also taken into account when deciding on clinical trial participation. The risk-benefit balancing act is also dependent on ongoing advances being made in the prevention and treatment of GVHD and in reducing the risk of post-ASCT relapse possibly through preemptive treatment. It is our opinion that genetic information not only is helpful in predicting survival outcome or risk of disease complications but also can be applied for monitoring treatment response as well as assessing risk of relapse after drug therapy or ASCT. To that end, it is imperative that clinical trials include laboratory correlative studies that are genetically pertinent.

Abbreviations and Acronyms: **ASCT** = allogeneic stem cell transplantation; **DIPSS** = Dynamic International Prognostic Scoring System; **ET** = essential thrombocythemia; **GVHD** = graft versus host disease; **IPSS** = International Prognostic Scoring System; **MPN** = myeloproliferative neoplasm; **PMF** = primary myelofibrosis; **PV** = polycythemia vera; **WHO** = World Health Organization

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