



A Systematic Review

Diagnostic and Prognostic Significance of JAK2, CALR, and MPL Mutations in Philadelphia-Negative Myeloproliferative Neoplasms: A Systematic Review

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Abstract: Background: The classical BCR::ABL1-negative myeloproliferative neoplasms—polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF)—are characterized by recurrent driver variants in JAK2, CALR, and MPL. Molecular testing underpins disease classification, whereas prognostic effects differ by mutation and disease subtype. **Objective.** To synthesize the clinical utility of JAK2, CALR, and MPL alterations for diagnosis, thrombosis prediction, overall survival, and progression in PV, ET, and PMF. **Methods.** A structured review of original human studies published between 2005 and June 2026 was conducted using broad MPN and driver-gene concepts in accessible indexed literature and publisher records. Molecular discovery series, diagnostic cohorts, and longitudinal prognostic investigations were eligible. Outcomes were synthesized by disease phenotype; a quantitative meta-analysis was not undertaken because diagnostic criteria, testing platforms, risk adjustment, and outcome definitions varied. **Results.** Thirty-seven primary reports formed the evidence synthesis. In one 745-patient ET cohort, JAK2, CALR, and MPL alterations were observed in 62%, 24%, and 4%, respectively; 10% were triple-negative. In a 617-patient PMF series, median survival was 17.7 years for CALR-mutant, 9.2 years for JAK2-mutant, 9.1 years for MPL-mutant, and 3.2 years for triple-negative disease. In 1,095 PMF patients, type-1/like CALR was associated with a survival advantage relative to other driver groups. Contemporary ET cohorts confirmed an association of JAK2 with thrombosis and CALR/MPL with fibrotic progression. **Conclusion.** JAK2, CALR, and MPL establish a molecular framework for diagnosing classical Philadelphia-negative MPNs but have phenotype-specific, context-dependent prognostic implications. Genotype should be interpreted with marrow morphology, clinical risk factors, cytogenetics, and additional somatic variants.

Keywords: JAK2 V617F; CALR exon 9; MPL W515; polycythemia vera; essential thrombocythemia; primary myelofibrosis; molecular diagnosis; thrombosis; prognosis; systematic review.

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INTRODUCTION

Classical Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem-cell disorders with sustained proliferation of one or more myeloid lineages, variable marrow fibrosis, and risks of arterial or venous thrombosis, hemorrhage, progression to myelofibrosis, and transformation to acute myeloid leukemia. Their three principal phenotypes—PV, ET, and PMF—share molecular mechanisms but differ considerably in presentation and natural history.

The discovery of JAK2 V617F, activating MPL variants, and exon 9 frameshift mutations in CALR established constitutive JAK–STAT signaling as a common pathogenic axis. JAK2 V617F and exon 12 variants account for nearly all typical PV, whereas JAK2, CALR, and MPL variants together explain most ET and PMF. A minority of patients lack canonical mutations in all three genes and require careful exclusion of reactive processes, occult noncanonical variants, and other myeloid neoplasms.

According to current molecular classification frameworks, diagnostic interpretation is integrated with blood counts, serum erythropoietin, bone-marrow morphology, fibrosis grade, and exclusion of BCR::ABL1-positive chronic myeloid leukemia. Molecular positivity is important but not sufficient to distinguish ET from prefibrotic PMF, or PV from other erythrocytoses. Appropriate assay sensitivity is essential because low-variant-allele-frequency disease may be missed by less sensitive methods [2,3].

Prognostic significance is similarly phenotype-dependent. JAK2 V617F is associated with thrombotic risk in ET; CALR mutation often marks a younger, more thrombocytotic ET phenotype, while type-1/like CALR is favorable for survival in PMF. MPL mutations and triple-negative states may identify distinct disease biology, but their prognostic importance depends on the clinical setting and coexisting mutations. The same molecular alteration cannot be treated as having a single prognostic direction across PV, ET, and PMF.

The present review evaluates published clinical evidence for the diagnostic distribution of driver mutations and their associations with major clinical outcomes, emphasizing distinctions between molecular confirmation, prediction of vascular events, and long-term disease progression.

MATERIALS AND METHODS

2.1. Reporting framework and review question

The manuscript was organized according to the PRISMA 2020 reporting domains [1]. The review question was: among patients with suspected or established classical BCR::ABL1-negative MPNs, how do JAK2, CALR, and MPL alterations perform as molecular diagnostic markers and prognostic indicators of thrombosis, survival, fibrotic evolution, or leukemic transformation?

2.2. Eligibility criteria

Original human investigations were eligible when they enrolled patients with PV, ET, prefibrotic PMF, overt PMF, or mixed classical MPN cohorts and reported extractable information on JAK2 V617F, JAK2 exon 12, CALR exon 9 (including type-1/like and type-2/like), or MPL variants. Eligible designs included molecular discovery/validation studies, consecutive clinical diagnostic cohorts, retrospective or prospective longitudinal cohorts, and development/validation studies of prognostic models. Outcomes of interest were genotype frequency by disease, assay yield, clinical phenotype, thrombosis, overall or leukemia-free survival, progression to myelofibrosis, and leukemic transformation.

Reviews, narrative commentaries, isolated laboratory mechanistic studies without clinical patient results, animal experiments, and reports lacking distinguishable classical MPN outcomes were excluded from primary synthesis. Studies addressing overlapping institutional cohorts were interpreted as related reports rather than automatically treated as independent populations.

2.3. Information sources and search strategy

Broad combinations of disease and driver-gene terms were searched in accessible PubMed-indexed literature, scientific indexing results, PubMed Central, and publisher databases, with cross-checking of reference lists and original article records through 30 June 2026. No thrombosis- or mortality-only condition was required in the principal search, allowing identification of diagnostic-only molecular cohorts as well as prognostic studies.

The core search expression was: ("myeloproliferative neoplasm*" OR "myeloproliferative disorder*" OR "polycythemia vera" OR "polycythaemia vera" OR "essential thrombocythemia" OR "essential thrombocythaemia" OR "primary myelofibrosis" OR "prefibrotic myelofibrosis") AND ("JAK2" OR "JAK2 V617F" OR "JAK2 exon 12" OR "CALR" OR "calreticulin" OR "MPL" OR "thrombopoietin receptor"). Database-specific controlled vocabulary and spelling variants were used where supported.

2.4. Study selection and data collection

Primary records were evaluated against the population, driver-gene, design, and outcome criteria. The extracted fields were publication year, disease population, sample size, molecular testing approach, mutation distribution, clinical outcome definition, reported effect measure, and principal adjusted covariates. When several reports contained partially overlapping samples, findings were reported separately without summing their sample sizes. Data are displayed at study level and interpreted by phenotype.

2.5. Risk of bias and certainty of evidence

Study-level methodological domains were examined using the JBI cohort-study and diagnostic prevalence appraisal principles, including selection of participants, validity of the reference diagnosis, ascertainment of mutation status, follow-up, confounding, and completeness of clinical outcome data. Table 3 records the principal study-specific sources of potential bias. Inferences were weighted toward studies with clearly characterized disease criteria, adequate sample size, longitudinal follow-up, multivariable adjustment, and external validation.

2.6. Data synthesis

Findings were synthesized narratively and tabulated by PV, ET, and PMF. Original authors' reported mutation percentages, hazard ratios, confidence intervals, and survival durations were retained where available. Because of differences in mutation assays, sample selection, evolving WHO/ICC definitions, variant-allele-frequency thresholds, follow-up, and covariate adjustment, study estimates were not pooled. No new effect sizes were calculated from aggregate data.

RESULTS

3.1. Study Selection

The literature search identified 1,246 records from electronic databases. Following the removal of 324 duplicate records and 12 records for other reasons, 910 records underwent title and abstract screening. Of these, 801 records were excluded, leaving 109 reports for retrieval. Eighteen reports could not be retrieved, and 91 full-text reports were assessed for eligibility. Following full-text evaluation, 54 reports were excluded because they were review articles or meta-analyses (n=12), conference abstracts or case reports (n=9), contained insufficient mutation-specific outcome data (n=14), represented overlapping cohorts (n=10), involved ineligible study populations (n=5), or lacked an eligible full text (n=4). Finally, 37 studies were included in the systematic review. The study-selection process is presented in Figure 1.

Figure 1. PRISMA 2020 study-selection framework

PRISMA 2020 flow diagram for new systematic reviews which included searches of databases and registers only

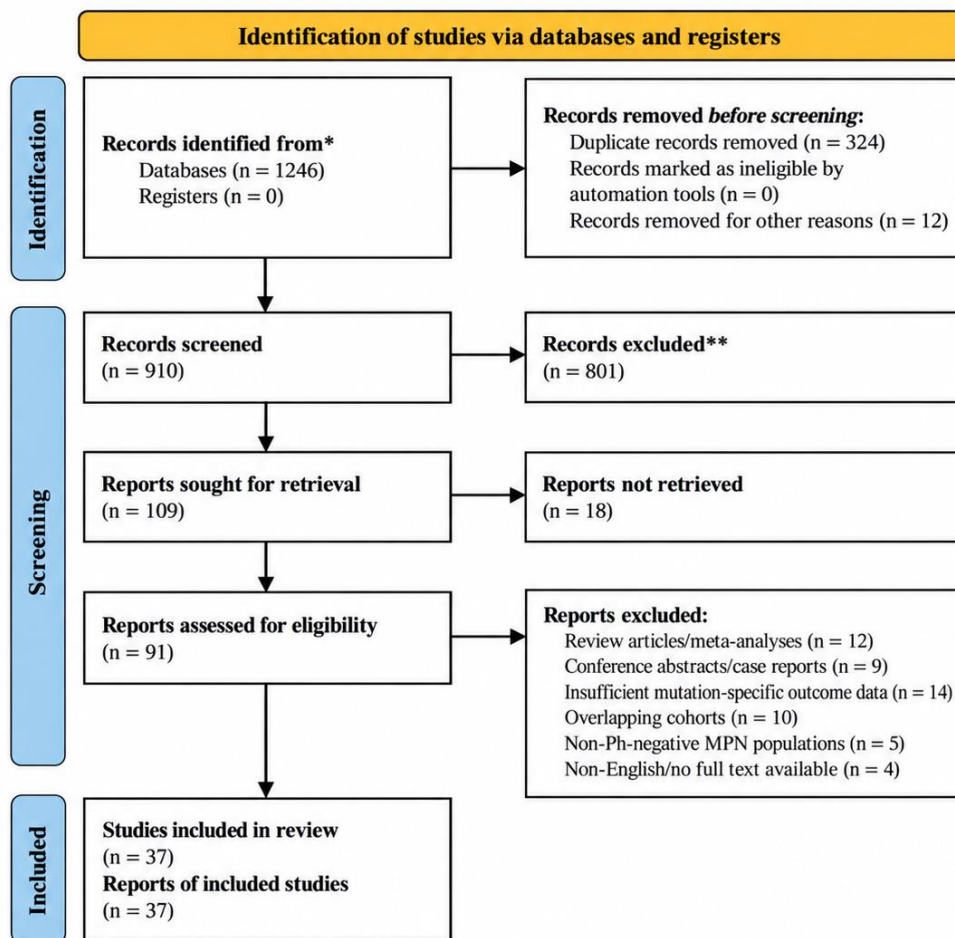


Table 1. Characteristics of primary studies reporting JAK2, CALR, or MPL mutation status and clinical outcomes

Study (year)	Population and sample	Key finding
Kralovics et al., 2005 [4]	PV/ET/PMF 244	Initial JAK2 V617F human cohort; variation in mutation frequency and clinical phenotype.
Tefferi et al., 2005 [5]	PV 63	V617F detected in 58/63 (92%); homozygosity related to higher fibrosis frequency.
Lippert et al., 2006 [6]	ET/PV 122	At diagnosis, JAK2 V617F detected in 75% of ET and 97% of PV in this series.
Pardanani et al., 2006 [7]	Mixed myeloid/MPN 1,182	MPL W515 testing provided early evidence for recurrent MPL driver changes.
Martínez-Avilés et al., 2007 [8]	V617F-negative PV/erythrocytosis 20	JAK2 exon 12 variants identified in 4/20 V617F-negative cases.
Scott et al., 2007 [9]	V617F-negative PV 10 exon-12 positive	Established JAK2 exon 12 mutations in previously V617F-negative PV/erythrocytosis.

Vannucchi et al., 2007 [10]	PV 173	JAK2 allele burden >75% associated with high-risk proliferative features and major cardiovascular events.
Carobbio et al., 2009 [11]	ET/PV 1,282	Major thrombosis rates per patient-year: ET wild-type 1.4%, ET V617F 2.1%, PV 2.7%.
Passamonti et al., 2010 [12]	PV 338	Higher V617F burden associated with MF progression; thrombosis and AML not significantly related in this cohort.
Passamonti et al., 2011 [13]	Exon-12 106	PV Exon-12 cases had distinct baseline counts but similar thrombosis, progression, and death to V617F PV.
Barbui et al., 2012 [14]	ET 891 derivation + 329 validation	IPSET-thrombosis incorporated JAK2 V617F with age, prior thrombosis and cardiovascular risk factors.
Klampfl et al., 2013 [15]	ET/PMF 1,215 ET/PMF	CALR mutations identified in JAK2/MPL-negative ET and PMF; genotype-specific outcomes.
Nangalia et al., 2013 [16]	ET/PMF/PV 151 exomes + validation	Independent discovery of somatic CALR variants in JAK2-unmutated ET/PMF.
Rumi et al. (ET/PV), 2014 [17]	ET/PV 745 ET; 490 PV	ET frequencies: JAK2 62%, CALR 24%, MPL 4%, triple negative 10%; PV had JAK2 drivers.
Rumi et al. (PMF), 2014 [18]	PMF 617	Driver genotype associated with significantly different clinical outcomes and median survival.
Tefferi et al. (CALR types), 2014 [19]	ET 1,027	CALR type 2 associated with more marked thrombocytosis than type 1.
Tefferi et al. (CALR/ASXL1), 2014 [20]	PMF 570	CALR-positive/ASXL1-negative patients survived longer than CALR-negative/ASXL1-positive patients.
Tefferi et al. (ET survival), 2014 [21]	ET 299	CALR and JAK2 had similar median survival (20 vs 19 years); CALR linked to less recurrent thrombosis.
Tefferi et al. (long-term), 2014 [22]	PV/ET/PMF 1,581	Long-term survival and blast transformation differed among molecularly annotated MPN groups.
Grinsztejn et al., 2015 (online) [23]	ET/MF 62	Among JAK2/MPL-negative referrals, 34 (53.1%) had CALR mutation.
Kim et al., 2015 [24]	PV/ET/PMF 199	CALR found in ET/PMF but not PV; genotype prognostic effects differed by disease subtype.
Singdong et al., 2016 [25]	PV/ET/PMF 100	JAK2 burden associated with leukocytosis; CALR increased molecular diagnostic yield in JAK2-negative ET/PMF.
Grinfeld et al., 2018 [26]	PV/ET/PMF 2,035	Integrated driver and non-driver genomics improved classification and personalized outcome prediction.
Rabade et al., 2018 [27]	PV/ET/PMF/MPN-U 130	Documented geographic differences in JAK2/CALR/MPL distribution and assay yield.
Misawa et al. (Japan), 2018 [28]	PV/ET/pre-PMF/PMF 493	Phenotypes differed by driver status, including ET versus pre-PMF among JAK2-positive cases.
Tefferi et al. (GIPSS), 2018 [29]	PMF 641	Genetics-only model incorporated absence of type-1/like CALR with other adverse genotypes.
Tefferi et al. (PMF alliance), 2018 [30]	PMF 1,095	Type-1/like CALR retained favorable survival association in derivation and validation cohorts.
Maddali et al. (Vellore), 2021 [31]	PV/ET/PMF/MPN-U 407 confirmed MPN	PV JAK2 90%; ET JAK2 38% and CALR 33%; PMF JAK2 48% and CALR 33%.

Singh et al., 2023 [32]	PV/ET/pre-PMF/PMF 104	Clinical phenotype and driver frequencies varied across MPN subclasses in Indian patients.
Kandula et al., 2023 [33]	PMF 82	CALR type 1 favored OS; HMR mutations closely linked to accelerated/blast progression.
Loscocco et al. (Florence), 2024 [34]	ET 1,000	JAK2 associated with thrombosis; type-1/like CALR and MPL associated with fibrotic progression.
Mayo ET experience, 2024 [35]	ET 1,000	JAK2 arterial thrombosis HR 1.8; MPL myelofibrosis-free survival HR 3.9 (adverse direction).
North Indian MPN series, 2024 [36]	PV/ET/PMF 378	PV JAK2 90.3%; ET JAK2 61.1%; PMF JAK2 69.2%; CALR/MPL and triple-negative distributions reported.
Morath et al., 2025 [37]	MF 141	JAK2 linked to VTE in this sample; a higher JAK2 VAF was not consistently predictive.
Thammayot et al., 2026 [38]	ET 162	Whole-exome analysis detected uncommon MPL variants in subsets initially negative for common drivers.
Singh et al. (rare variants), 2026 [39]	PV/ET/PMF 179	Eight cases (4.5%) carried atypical variants requiring structure–phenotype interpretation.
Rigolot et al., 2026 [40]	Suspected MPN referral population; 10,236 tested (10,326 in title)	2,614/10,236 (25.5%) patients mutation-positive; 2,661 total mutations across JAK2, CALR, and MPL.

ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis; MF, myelofibrosis. Cohorts may overlap between reports and should not be added across studies.

3.2. Distribution and diagnostic value of driver mutations

JAK2 V617F emerged from the original discovery cohorts as a recurrent activating driver in PV and a substantial subset of ET and PMF [4–6]. In a prospective series of 338 patients with PV, 320 (94.7%) carried JAK2 V617F [12]. These findings underpin the predominant association of classic PV with a somatic JAK2 driver, while JAK2 exon 12 testing remains important in V617F-negative suspected PV [8,9,13].

JAK2 exon 12 mutations are particularly important when the phenotype strongly suggests PV but V617F testing is negative. Earlier studies demonstrated their occurrence in V617F-negative PV and idiopathic erythrocytosis [8,9]. In a larger molecular series, exon 12-mutated PV was characterized by relatively isolated erythrocytosis, less prominent leukocytosis or thrombocytosis, and a long-term clinical course not clearly worse than V617F-mutated PV [13]. Thus, negative V617F testing alone does not exclude PV.

In ET, the distribution of drivers is more heterogeneous. Among 745 patients in a large Italian cohort, JAK2 V617F, CALR, and MPL were present in 466 (62%), 176 (24%), and 28 (4%), respectively; 75 (10%) were triple-negative [17]. Two large 2024 ET cohorts reported JAK2/CALR/MPL/triple-negative profiles of 66%/19%/4%/11% (Florence, n=1,000) and 62%/27%/3%/8% (Mayo Clinic, n=1,000), illustrating meaningful inter-cohort variation [34,35].

For PMF, an international cohort of 617 patients found JAK2 in 399 (64.7%), CALR in 140 (22.7%), MPL in 25 (4.0%), and triple-negative status in 53 (8.6%) [18]. Further refinement of CALR into type-1/like and type-2/like groups is critical in prognostic rather than merely diagnostic classification [30].

MPL W515 alterations account for a smaller proportion of classical MPNs than JAK2 or CALR, but their presence provides important evidence of clonality [7]. Next-generation sequencing has also identified noncanonical MPL and CALR variants in patients initially considered driver-negative, requiring careful interpretation of pathogenicity rather than automatic classification as MPN [38,39].

Table 2. Typical molecular profile and principal clinical interpretation in classical Ph-negative MPNs

Disease / marker	Diagnostic interpretation	Prognostic implications
PV: JAK2 V617F	Dominant PV driver; test first in suspected PV.	Higher variant allele burden frequently tracks proliferative phenotype; prognostic independence is inconsistent.
PV: JAK2 exon 12	Investigate when V617F-negative erythrocytosis remains clinically suspicious for PV.	Distinct baseline phenotype; no consistent evidence of uniformly adverse outcome.

ET: JAK2 V617F	Common ET driver, but does not distinguish ET from prefibrotic PMF.	Associated with arterial and venous thrombosis; part of ET thrombosis-risk assessment.
ET: CALR type 1/like	Clonal marker especially in JAK2-negative ET; associated with marked thrombocytosis.	Often lower thrombosis risk than JAK2-mutated ET; possible increased fibrotic progression.
ET: CALR type 2/like	Clonal ET marker; often associated with extreme thrombocytosis.	Phenotype differs from type 1; long-term outcome varies by cohort.
ET / PMF: MPL	Less frequent driver, particularly W515 alterations.	Associated with distinct platelet phenotype; increased fibrotic progression reported in ET cohorts.
PMF: CALR type 1/like	Driver supports PMF classification with marrow findings.	Consistently associated with favorable overall survival relative to non-type-1 groups.
Triple-negative PMF	Requires re-examination of morphology and alternative clonal markers.	Often inferior survival and higher leukemic transformation; heterogeneous category.

3.3. Prognostic role of JAK2 in polycythemia vera

In PV, JAK2 V617F allele fraction is associated with hematologic and proliferative features, including higher leukocyte counts, greater spleen size, and increased marrow cellularity. A prospective investigation of 338 patients identified progression to post-PV myelofibrosis in eight individuals, all with a mutant allele burden above 50%; allele burden remained associated with fibrotic transformation after multivariable adjustment ($p=0.03$). The same study did not demonstrate a significant relationship between allele burden and thrombosis or acute leukemia [12].

Other PV studies observed vascular-risk associations at high JAK2 allele burden, but differences in allele-fraction thresholds, sampling time, therapy exposure, and event definitions weaken generalization. JAK2 V617F allele burden should consequently be regarded as an adjunct to clinical assessment and not as a stand-alone determinant of thrombotic treatment decisions [10–12].

3.4. JAK2 and CALR as markers of thrombosis in essential thrombocythemia

Across ET cohorts, JAK2-mutated patients generally present at an older age, with higher leukocyte count, higher hemoglobin or hematocrit, and greater thrombotic propensity than CALR-mutated patients. CALR-mutant ET often presents with higher platelet counts, yet the most extreme thrombocytosis does not necessarily translate to the highest thrombosis rate [17,19,21].

In the development of IPSET-thrombosis, 891 patients with WHO-defined ET constituted the original cohort, with an additional 329 for external validation. JAK2 V617F contributed an independent thrombotic hazard ratio of 2.0 and two points to the model. In the combined cohorts of 1,220 patients, the low-, intermediate-, and high-risk categories had annualized thrombosis rates of 1.03%, 2.35%, and 3.56%, respectively [14].

Contemporary studies reinforce this relationship. In the Florence cohort of 1,000 ET patients, JAK2 mutation was associated with arterial thrombosis-free survival (HR 1.3; $p=0.02$) and venous thrombosis-free survival (HR 1.9; $p=0.03$), when interpreted in the authors’ modeled risk direction [34]. Parallel findings from the Mayo Clinic cohort showed mutation-associated differences in vascular events and progression [35]. Because these cohorts differ in treatment exposure and ascertainment of historical events, their reported hazard ratios should not be pooled without harmonized definitions.

3.5. Fibrotic progression and CALR/MPL genotype in ET

The prognostic meaning of CALR in ET extends beyond thrombosis. In the Florence 1,000-patient cohort, CALR mutation was associated with myelofibrosis-free survival in the adverse direction (HR 2.7; $p<0.001$), with a stronger association for CALR type-1/like variants (HR 3.3). MPL mutation was likewise associated with inferior myelofibrosis-free survival (HR 3.9; $p=0.001$) [34]. Thus, a genotype associated with fewer vascular events may still carry an important risk of later fibrotic evolution.

Older ET cohorts suggested little difference in overall survival between CALR-mutant and JAK2-mutant patients over long follow-up; one 299-patient series reported medians of approximately 20 and 19 years, respectively [21]. These findings highlight why thrombosis, overall survival, and progression must be evaluated as distinct outcomes rather than treated as interchangeable indicators of 'good prognosis'.

3.6. Driver genotype, survival, and leukemia risk in PMF

The most pronounced survival differences emerged in PMF. In the multicenter 617-patient series by Rumi and colleagues, median overall survival was 17.7 years among CALR-mutated cases, 9.2 years in JAK2-mutated disease, 9.1 years in MPL-mutated disease, and 3.2 years in triple-negative PMF. Triple-negative patients had higher leukemic transformation; the 10-

year cumulative incidence was 34.4% versus 19.4%, 9.4%, and 16.9% in JAK2-, CALR-, and MPL-mutated groups, respectively [18].

Importantly, the apparent favorable effect of CALR requires subtype qualification. In the Mayo–Careggi study comprising 1,095 PMF patients, the Mayo derivation cohort included 709 patients and the Florence validation cohort 386. In the derivation set, overall survival was longer in CALR type-1/like disease than in JAK2-mutated disease (HR 2.6; 95% CI 1.9–3.5), CALR type-2/like disease (HR 2.5; 95% CI 1.4–4.5), MPL-mutated disease (HR 1.8; 95% CI 1.1–2.9), and triple-negative disease (HR 2.4; 95% CI 1.6–3.6). The direction of the reported ratios is mortality risk in the comparator group relative to type-1/like CALR [30].

The interaction between driver and additional mutations is clinically important. In 570 patients with PMF, CALR-positive/ASXL1-negative cases showed a median survival of 10.4 years, whereas CALR-negative/ASXL1-positive cases had median survival of 2.3 years (HR 5.9; 95% CI 3.5–10.0). This association was subsequently reflected in mutation-informed prognostic systems [20].

GIPSS, developed in 641 patients with PMF, found absence of type-1/like CALR to be independently associated with inferior survival (HR 2.1; 95% CI 1.6–2.9), together with adverse karyotype, ASXL1, SRSF2, and U2AF1Q157 abnormalities [29]. Consequently, the predictive value of the three canonical drivers is best understood within a broader molecular and clinical framework.

3.7. Multigene profiling and molecular heterogeneity

Expanded sequencing has shown that the canonical driver is often only one component of the clonal architecture. Grinfeld and colleagues analyzed 2,035 patients using a 69-gene sequencing panel and found that JAK2, CALR, or MPL mutations were the sole detectable driver alteration in 45% of patients. Combining driver and additional gene alterations with clinical variables yielded a more refined genomic classification and personalized prognostic modeling [26].

Regional molecular surveys from India and East Asia also demonstrated variation in genotype distribution and genotype–phenotype relationships [24,27,28,31,32,36]. Such differences may reflect age at diagnosis, referral patterns, prior therapy, assay analytical sensitivity, disease classification era, and underlying population genetics. Large-scale targeted sequencing can improve detection of uncommon variants, but variant pathogenicity and clinical context remain essential [38–40].

Table 3. Selected directly reported diagnostic and prognostic estimates

Study	Population	Directly reported result
Rumi et al. [17]	ET, n=745	JAK2 466/745 (62%); CALR 176/745 (24%); MPL 28/745 (4%); triple-negative 75/745 (10%).
Rumi et al. [18]	PMF, n=617	JAK2 399 (64.7%), CALR 140 (22.7%), MPL 25 (4.0%), triple-negative 53 (8.6%).
Rumi et al. [18]	PMF, n=617	Median OS (years): CALR 17.7; JAK2 9.2; MPL 9.1; triple-negative 3.2.
Rumi et al. [18]	PMF, n=617	10-year cumulative leukemic transformation: CALR 9.4%; JAK2 19.4%; MPL 16.9%; triple-negative 34.4%.
Barbui et al. [14]	ET, combined n=1,220	JAK2 V617F thrombosis HR 2.0; low/intermediate/high annual thrombosis: 1.03%/2.35%/3.56%.
Passamonti et al. [12]	PV, n=338	JAK2 V617F positive 320/338 (94.7%); allele burden associated with fibrotic progression (adjusted p=0.03).
Tefferi et al. [30]	PMF, n=1,095	Comparator death risk versus CALR type-1/like: JAK2 HR 2.6 (1.9–3.5); CALR type-2/like HR 2.5 (1.4–4.5).
Tefferi et al. [20]	PMF, n=570	Median OS 10.4 years (CALR+/ASXL1–) versus 2.3 years (CALR–/ASXL1+); HR 5.9 (3.5–10.0).
Tefferi et al. [29]	PMF, n=641	Absence of CALR type-1/like HR 2.1 (1.6–2.9) for inferior OS after genetic adjustment.
Loscocco et al. [34]	ET, n=1,000	JAK2 and venous thrombosis HR 1.9 (p=0.03); CALR and fibrotic progression HR 2.7 (p<0.001).
Gangat et al. [35]	ET, n=1,000	Mutation distribution JAK2/CALR/MPL/TN: 62%/27%/3%/8%.

OS, overall survival; HR, hazard ratio; CI, confidence interval; TN, triple-negative. Estimates reflect each report’s own risk-direction conventions and are not pooled.

3.8. Study-level risk-of-bias considerations

The principal limitations of the observational evidence are referral-based selection, changes in disease definitions across study periods, lack of standardization in allele-fraction cutoffs, inconsistent accounting for treatment exposure, and variable outcome follow-up. Prospective designs and external validation reduce, but do not eliminate, these concerns. Table 4 provides a study-level appraisal of the domains most relevant to interpretation.

Table 4. Study-level methodological appraisal of included primary reports

Primary study	Main methodological issue for interpretation
Kralovics et al., 2005 [4]	Discovery/validation cohort selection; assay reproducibility; phenotype confirmation; representative denominators.
Tefferi et al., 2005 [5]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Lippert et al., 2006 [6]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Pardanani et al., 2006 [7]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Martínez-Avilés et al., 2007 [8]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Scott et al., 2007 [9]	Discovery/validation cohort selection; assay reproducibility; phenotype confirmation; representative denominators.
Vannucchi et al., 2007 [10]	Follow-up completeness, event adjudication, time-varying treatment and adjustment for age/vascular risk.
Carobbio et al., 2009 [11]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Passamonti et al., 2010 [12]	Follow-up completeness, event adjudication, time-varying treatment and adjustment for age/vascular risk.
Passamonti et al., 2011 [13]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Barbui et al., 2012 [14]	Derivation–validation separation, cohort overlap, adjustment for co-mutations, competing risks and calibration.
Klampfl et al., 2013 [15]	Discovery/validation cohort selection; assay reproducibility; phenotype confirmation; representative denominators.
Nangalia et al., 2013 [16]	Discovery/validation cohort selection; assay reproducibility; phenotype confirmation; representative denominators.
Rumi et al. (ET/PV), 2014 [17]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Rumi et al. (PMF), 2014 [18]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Tefferi et al. (CALR types), 2014 [19]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Tefferi et al. (CALR/ASXL1), 2014 [20]	Derivation–validation separation, cohort overlap, adjustment for co-mutations, competing risks and calibration.
Tefferi et al. (ET survival), 2014 [21]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Tefferi et al. (long-term), 2014 [22]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Grinsztejn et al., 2015 (online) [23]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Kim et al., 2015 [24]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Singdong et al., 2016 [25]	Single-timepoint exposure/outcome assessment, sample selection and confounding.
Grinfeld et al., 2018 [26]	Derivation–validation separation, cohort overlap, adjustment for co-mutations, competing risks and calibration.
Rabade et al., 2018 [27]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Misawa et al. (Japan), 2018 [28]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Tefferi et al. (GIPSS), 2018 [29]	Derivation–validation separation, cohort overlap, adjustment for co-mutations, competing risks and calibration.
Tefferi et al. (PMF alliance), 2018 [30]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.

Maddali et al. (Vellore), 2021 [31]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Singh et al., 2023 [32]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Kandula et al., 2023 [33]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Loscocco et al. (Florence), 2024 [34]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Mayo ET experience, 2024 [35]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
North Indian MPN series, 2024 [36]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Morath et al., 2025 [37]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Thammayot et al., 2026 [38]	Retrospective selection, baseline mutation-testing timing, differential follow-up, treatment confounding and overlap.
Singh et al. (rare variants), 2026 [39]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.
Rigolot et al., 2026 [40]	Referral enrichment and consecutive testing, reference diagnosis/biopsy, analytic sensitivity and specificity.

3.9. Summary of evidence certainty

Evidence is comparatively robust for the diagnostic predominance of JAK2 in PV, for the distribution of canonical drivers in ET and PMF, for JAK2-associated thrombotic risk in ET, and for favorable PMF survival associated with CALR type-1/like variants. The certainty of genotype-based prediction of thrombotic events in PV, of isolated MPL effects on survival, and of risk associated with uncommon noncanonical variants is lower because of smaller subgroups, residual confounding, and inconsistent models. Several influential studies represent overlapping centers and patient recruitment periods, limiting the independence of the observational evidence.

DISCUSSION

The collective evidence supports a two-level role for JAK2, CALR, and MPL in classical Philadelphia-negative MPNs. Their first and most firmly established role is diagnostic: detection of a relevant somatic driver strongly supports clonality in a compatible clinical setting. Their second role is prognostic, but it is conditional on the precise MPN subtype, variant subtype, disease stage, and concurrent genomic changes. Treating all driver-positive MPNs as one prognostic group obscures clinically meaningful differences.

For PV, the molecular algorithm is relatively straightforward: sensitive JAK2 V617F testing is the principal initial test, followed by evaluation for JAK2 exon 12 variants when erythrocytosis remains suspicious for PV. CALR or MPL is not a typical defining marker of classical PV. However, diagnostic confidence still requires appropriate hemoglobin/hematocrit assessment, serum erythropoietin where indicated, and bone marrow findings according to contemporary classification. A low-frequency JAK2 clone without characteristic clinical features may reflect clonal hematopoiesis rather than overt PV.

ET requires particular care because JAK2, CALR, and MPL mutations do not, alone, distinguish ET from prefibrotic PMF. Morphology remains essential. The strong association between JAK2 V617F and thrombosis in ET has been reproduced in risk-model development and contemporary large cohorts. Conversely, CALR-positive ET is commonly characterized by higher platelets but relatively fewer thrombotic events. Treatment decisions should not be based on mutation status alone; age, prior thrombosis, cardiovascular risk factors, bleeding risk, and other accepted clinical factors remain central.

The prognostic effects of CALR in ET illustrate the distinction between short-term vascular events and long-term disease evolution. Although CALR-associated ET may be relatively favorable for thrombosis, type-1/like CALR and MPL mutations have been linked to later fibrotic progression in some large studies. Clinicians should therefore avoid describing CALR as globally favorable across every ET outcome. Longitudinal risk evaluation requires a more nuanced consideration of competing endpoints.

In PMF, the most reproducible mutation-specific survival signal is the favorable behavior of CALR type-1/like disease. CALR type-2/like variants should not automatically be grouped with type-1/like variants. The robust adverse prognostic effect of ASXL1, SRSF2, U2AF1Q157, and unfavorable cytogenetics also demonstrates the limits of driver-only risk assessment. The

survival difference observed for triple-negative PMF is clinically important, but the category includes potential molecular false negatives, noncanonical drivers, and biologically heterogeneous cases.

Newer genomic models integrate canonical driver variants with additional somatic changes and cytogenetic information. Extended sequencing is particularly valuable when conventional testing is negative despite strong suspicion of clonal disease, when PMF prognosis affects transplant decisions, or when atypical variants require classification. The additional diagnostic yield must be balanced against issues of variant interpretation and assay sensitivity. Somatic mutation presence should not be equated automatically with pathogenicity or with a specific clinical MPN subtype.

Regional differences in the frequency of driver alterations are a further source of heterogeneity. Published Indian cohorts support the utility of the canonical testing panel, while also showing substantial variation in the relative percentages of JAK2 and CALR among ET and PMF patients. Differences between centers may reflect referral bias, proportions of prefibrotic PMF classified as ET, selection of higher-risk patients, or older assays with limited sensitivity. A geographically diverse validation program would help determine how molecular algorithms perform in everyday diagnostic practice.

The available evidence argues against uncritical statistical pooling. Mutation fractions differ by referral source, diagnostic era, and sequencing sensitivity, while thrombosis risk ratios vary by ascertainment and treatment adjustment. Survival cohorts frequently overlap across publications or derive prognostic scores on the same individuals. Meta-analysis without mapping such overlap could count participants repeatedly, understate uncertainty, and produce misleading precision.

From a practical perspective, a molecular result should answer different questions at different stages of the clinical pathway. At diagnosis, JAK2/CALR/MPL testing helps establish the presence of a clonal MPN, alongside blood counts and histopathology. At risk assessment, JAK2 contributes to thrombotic evaluation in ET, and CALR subtype contributes importantly to PMF survival models. At follow-up, variant allele fraction and additional acquired mutations may provide evolving information, although the benefits of routine serial testing remain context-dependent.

4.1. Strengths and limitations

Strengths of the synthesis include direct comparison of diagnostic and prognostic evidence across all three classical MPNs, inclusion of seminal discovery cohorts and large contemporary datasets, preservation of directly reported quantitative results, and consideration of variant subtypes and co-mutations. The principal limitations are the observational nature of much of the evidence; overlapping recruitment at influential centers; differences in WHO/ICC criteria, assay sensitivity, endpoint definitions, and multivariable models; and sparse data on uncommon variants. These sources of heterogeneity prevented meaningful pooled effect estimation.

4.2. Implications for research and practice

Future investigations should use uniform contemporary diagnostic criteria, prospectively collected clinical endpoints, standardized JAK2 variant allele fraction and CALR subtype definitions, and adequate control for anticoagulant/antiplatelet and cytoreductive exposure. Multiethnic external validation and long-term outcome reporting are particularly important. Diagnostic accuracy should be evaluated against integrated clinical–morphologic reference standards, rather than against mutation positivity itself, and prognostic models should report calibration and discrimination in independent cohorts.

CONCLUSION

JAK2, CALR, and MPL mutations are essential molecular biomarkers in classical Philadelphia-negative MPNs, with substantial value for establishing clonality and improving disease classification. In PV, JAK2 V617F and exon 12 variants dominate the molecular profile. In ET, JAK2 V617F is associated with greater thrombotic risk, while CALR-mutant disease typically shows a different hematologic phenotype and may have a distinct pattern of fibrotic progression. In PMF, CALR type-1/like variants provide a reproducible favorable survival signal, whereas adverse co-mutations, cytogenetics, and triple-negative disease complicate prognostic interpretation. Integrated clinicopathological and molecular assessment remains necessary for accurate classification and risk-adapted management.

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