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Association of JAK2, CALR, and MPL Driver Mutations with NF-κB/p65 Expression in Iraqi Patients with Philadelphia Chromosome-Negative Myeloproliferative Neoplasms

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Abstract

Background: Myeloproliferative neoplasms (MPNs) are shaped by both oncogenic mutations and chronic inflammation. Nuclear Factor-kappa B (NF-κB) is a central regulator of inflammation, yet its role in defining the clinical profile of Middle Eastern its relationship to molecular drivers and clinical outcomes in an Iraqi MPN cohort.
Methods: We conducted a combined prospective and retrospective analysis of 110 patients with BCR-ABL1-negative MPNs (40 PV, 40 ET, 30 PMF) alongside 40 age- and sex-matched healthy controls. Peripheral blood was tested for JAK2, CALR, and MPL mutation ns using real-time PCR. NF-κB/p65 mRNA expression was quantified by RT-qPCR.
Results: Median age at diagnosis (55-58 years) was younger than Western cohorts, consistent with regional trends. JAK2 (V617F) was the most frequent mutation (61.8%). NF-κB/p65 expression was significantly elevated across all MPN subtypes compared with controls ($p < 0.001$), following a severity gradient: PMF (3.6-fold) > PV (2.7-fold) > ET (2.2-fold). The highest level of activation was observed in JAK2 mutation cases (3.2-fold increased), but elevated NF-κB levels were also observed in CALR/MPL cases (2.4-fold increased) and patients with triple-negative results (2.6-fold increased). Clinically, elevated NF-κB levels were closely associated with splenomegaly ($p < 0.01$) and thrombosis ($p < 0.001$).
Conclusions: The transcription factor NF-κB/p65 links malignant proliferation and inflammation in bone marrow proliferative diseases, with its activation accurately reflecting disease severity and vascular risk. Therefore, it can be considered a biomarker and therapeutic target for such diseases.

Keywords:
Myeloproliferative Neoplasms, Drive Genes, NF-κB/p65, Iraq.

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Introduction

Myeloproliferative neoplasms (MPNs) are hematopoietic stem cell proliferative disorders characterized by persistent overproduction of myeloid cells. These tumors are commonly associated with coagulation

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and bleeding complications, progressive myelofibrosis, and an increased risk of transformation to acute leukemia (Tefferi and Barbui, 2020; Grinfeld et al., 2025). The major Philadelphia chromosome-negative MPNs-polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) share many clinical and pathological features (Gerds et al., 2022). However, there is considerable biological diversity among these diseases, suggesting that factors other than the underlying genetic mutations, including additional molecular alterations and inflammatory processes, play a major role in shaping the disease phenotype and progression (Baumeister et al., 2021).

The molecular landscape of myeloproliferative diseases is largely shaped by reciprocal, exclusive, inducible mutations in the JAK2, CALR, and MPL genes, leading to sustained activation of cytokine receptor signaling and subsequent pathways that promote cell proliferation and survival. Among these mutations, the most common is the JAK2 V617F mutation, which enhances cytokine-independent signaling through sustained activation of the JAK-STAT pathway (Morsia et al., 2022; Pan et al., 2026). This gain-of-function mutation causes cytokine-independent signaling, resulting in uncontrolled myeloproliferation and altered differentiation patterns. Notably, the mutation type and allelic load influence the disease phenotype and severity, highlighting the gene dose-dependent enhancement of cancer signaling (Pan et al., 2026). The mutation is found in almost all cases of polycythemia vera and in a large proportion of patients with essential primary myelofibrosis and thrombocythemia (Morsia et al., 2022).

The identification of exon 9 mutations in the CALR gene and exon 10 mutations in the MPL gene has improved the molecular classification of myeloproliferative disorders, particularly in patients without the JAK2 mutation, and highlighted the pivotal role of disrupted thrombopoietin receptor signaling in disease progression (Costa and Breccia, 2025; Stivala and Meyer, 2021). Large-scale genomic analyses indicate that these three driver mutations JAK2, CALR, and MPL are largely mutually exclusive and collectively explain most classic myeloproliferative disorders, influencing not only clinical features and prognosis but also treatment response (Luque et al., 2023). The phenotypic and clinical variability of patients with identical driver mutations, however, indicates that other molecular processes and/or factors may also be involved in the severity of symptoms, disease progression and/or clonal expansion and suggests a critical need to investigate region (phenotype) specific molecular drivers which can modify the phenotype and disease severity.

Chronic inflammation is considered an increasingly important major factor in the development of myeloproliferative disorders. Patients often exhibit elevated levels of circulating inflammatory cytokines, such as interferons, tumor necrosis factor-alpha (TNF-α) and various interleukins, which contribute to hematopoietic dysplasia, systemic symptoms and remodeling of the bone marrow microenvironment (Fisher et al., 2021). These inflammatory signals are not merely a reactive byproduct of disease; they actively promote malignant cell survival and may occur even before the onset of overt clonal dominance (Fisher et al., 2021; Hermouet, 2023). Nuclear factor kappa B (NF-κB) is a central link between cancer signaling and inflammation in myeloproliferative disorders. It is a transcription factor complex that regulates genes stress responses, involved in immune activation and cell survival (Kagoya et al., 2014; Fisher et al., 2023).

The NF-κB family consists of five structurally similar transcription factors:

c-REL, RELA (p65), NF-κB1 (p50/p105), NF-κB2 (p52/p100) and RELB. These are encoded by genes located at distinct chromosomal loci, including NFKB1 (4q24), RELA (11q13.1), RELB (19q13.32), NFKB2 (10q24.32) and REL (2p16.1) (Msweli et al., 2024). These subunits can form heterologous or homologous dichotomies, which are typically retained in the cytoplasm through interactions with IκB inhibitory proteins. Classical NF-κB activation occurs when the IκB kinase complex phosphorylates IκBα, leading to its targeting for proteasome lysis and allowing translocation of RELA or c-REL dimers to the nucleus. In contrast, the non-classical pathway involves NFKB2 processing and the formation of RELB-p52 complexes (Collins et al., 2016). In myeloproliferative disorders, NF-κB activation is largely mutation-independent and is instead sustained by oncogenic JAK-STAT signaling and chronic exposure to inflammatory cytokines such as IL-1β and TNF-α (Sullivan and Fleischman, 2024). Similar activation patterns are observed in other malignant myelomas, chronic inflammatory conditions, and autoimmune disorders, highlighting the central role of NF-κB as a mediator of pathogenic inflammation and cell survival across diverse disease contexts (Hoffmann et al., 2025).

The persistence of myeloproliferative disorders is not solely determined by genetic factors, but also results from a strong synergy between cellular signaling pathways (Morsia et al., 2022). Although direct mutations in NF-κB are rare, this pathway remains continuously active through a self-sustaining feedback loop. Cancer mutations, such as JAK2V617F, may ignite the initial spark, but circulating inflammatory cytokines keep the disease alive. By linking JAK-STAT signaling to chronic

inflammatory signals, NF-κB helps create a pro-inflammatory environment in which malignant cells not only survive but actively outcompete and suppress normal hematopoiesis (Morsia et al., 2022; Pan et al., 2026). With molecular profiling coupled with cytokine bio-profiling, a more dynamic prognostic framework has thus been provided. These sustained levels of hyper-inflammatory cytokines create a selective environment for the growth of the mutant hematopoietic stem cells (HSC) and establish a self-reinforcing sanctuary that leads to the persistent growth of the mutant clones and resistance to standard targeted therapies (Soyfer and Fleischman, 2023).

Despite the important role of NF-κB in MPN pathogenesis and therapy resistance, knowledge so far is largely from Western populations, resulting in a substantial lack of knowledge about the activation of NF-κB in Middle Eastern populations. No study has been performed to date to comprehensively assess the expression of NF-κB signaling molecules in relation to driver mutations (JAK2, CALR, MPL) and systemic inflammation in an Iraqi MPN cohort. This pathway is very important to study, given the unique genetic background, distinct environmental exposures, and regional variability in disease presentation in Iraq. The present study fills in the regional understanding of differential expression of Iraqi MPN patients have NF-κB subunits. The mapping of the interplay between oncogenic mutations, inflammatory microenvironments and NF-κB dysregulation not only offer new diagnostic and prognostic value for NF-κB in a previously uncharacterized population of MPNs but also increases the global comprehensiveness of the understanding of MPN biology.

Materials and Methods

Study Design and Patient Selection

This retrospective observational study, conducted from January 2022 to December 2025, evaluated a cohort of 110 adult patients with classical Philadelphia chromosome-negative MPNs. The cohort included patients with polycythemia vera (n=40), essential thrombocythemia (n=40), and primary myelofibrosis (n=30), diagnosed according to WHO criteria and the International Classification of Diseases (ICD) by specialist physicians. A group of 40 age- and sex-matched healthy volunteers served as a comparator population (total N=150). Eligible patients were 18 years of age or older and had available peripheral blood samples collected in EDTA obtained either at the time of diagnosis or during routine follow-up, preferably before the initiation of disease-modifying therapy. Patients were excluded if they had BCR/ABL1-positive disease, other hematologic malignancies, active infectious or inflammatory conditions, or samples of insufficient quality. Healthy control subjects had no history of hematologic malignancies, autoimmune disorders, chronic inflammatory diseases, or cancer, and demonstrated normal blood counts at the time of sampling. Individuals receiving immunosuppressive or cytotoxic treatments were excluded. All samples were processed immediately after collection or stored at -20 °C until analysis. Ethical approval was obtained Written informed consent was obtained from all participants prior to inclusion in the study.

Clinical and Laboratory Data Collection

Demographic and clinical data were systematically collected for all patients at the time of sample acquisition. Recorded variables included age, sex, disease subtype, and the presence or absence of splenomegaly, as determined by physical examination and/or imaging studies. A documented history of clotting or bleeding events was also recorded. Hematological parameters were obtained from a complete blood count performed concurrently with sample collection and included hemoglobin levels, white blood cell count and platelet count. These clinical and laboratory data were subsequently used for correlative analyses with molecular findings.

Genomic DNA Extraction from Whole Blood

Genomic DNA was isolated from peripheral blood samples using the solution-based Blood DNA Preparation Kit (cat. PP-205S; Jena Bioscience GmbH, Germany) according to the manufacturer's instructions. In brief, whole blood was analyzed under chaotic conditions optimized for nucleic acid release, then protein-digested to remove cellular proteins and increase the amount of DNA. The resulting extract was then processed using the group's proprietary purification matrix, enabling the selective binding of genomic DNA. After several washing steps to remove residual contaminants, the DNA was extracted in a low-salt solution. DNA concentration and purity were assessed using N50/N60 microvolume spectroscopy (Implen, GmbH, Germany) and DNA integrity was verified by agarose gel electrophoresis (Guha et al., 2018; Lee and Tripathi., 2020).

Real-Time PCR Driver Mutations Detection

To characterize the molecular structure of the cohort, genomic DNA was extracted using a blood DNA preparation kit (Jena Bioscience GmbH, Germany) according to the manufacturer's instructions. Driver mutation assays were performed using quantitative polymerase chain reaction (PCR) (MPN Assay Kit; SNP Biotechnology, Turkey). The assay used four multiplex reaction kits to detect variants in exons 12 and 14 (V617F) of the JAK2 gene, exon 9 of the CALR gene, and exon 10 of the MPL gene. Alleles were discriminated using probes labeled with FAM, HEX, and Texas Red, while the internal assay was confirmed by co-amplification of the prothrombin (FII) gene, which was detected in the CY5 channel. Each reaction consisted of 20 µL of the main mix and 5 µL of genomic DNA, followed by short centrifugation. Real-time PCR was performed on a Bio-Rad CFX96 Touch Real-Time PCR Detection System (bIOrAD using an initial denaturation at 96 °C for 1 min, followed by 32 cycles of 96 °C for 2 s and 60 °C for 30 s. Real-time PCR was performed on a four-channel system with initial denaturation at 96 °C for 1 min, then 32 cycles of 96 °C for 2 s and 60 °C for 30 s. Fluorescence signals were recorded at each cycle, and mutation status was determined by threshold cycle analysis. Run validity was confirmed by appropriate internal control amplification, and mutations were considered positive when dye-specific signals exceeded the defined threshold (Al-Dayyeni et al., 2023).

Total RNA Isolation

Total RNA was extracted from patient blood samples using the column-based Total RNA Purification Kit (cat. PP-210L; Jena Bioscience GmbH, Germany) according to the manufacturer's instructions. Blood cells were lysed in a chaotic buffer solution to release RNA and inactivate ribonucleases (RNases). The extract was then placed on rotating silica columns to selectively bind the RNA. After sequential washing to remove proteins, genomic DNA, and other contaminants, the RNA was extracted in RNase-free water. RNA concentration and purity were assessed using spectroscopy, and its integrity was confirmed by agarose gel electrophoresis. Residual genomic DNA was removed using a genomic DNA removal kit (product number PP-219; Jena Bioscience GmbH, Germany) by deoxyribonucleic acid (DNase) treatment under optimized conditions (Zeng et al., 2024; Nguyen et al., 2024).

Transcriptional Analysis of NF-κB/p65

To investigate the transcriptional mechanisms underlying the inflammatory milieu of the MPNs patients, mRNA expression of NF-κB/p65 subunit was analyzed. Total RNA was isolated from peripheral blood samples using the Total RNA Purification Kit - Column Kit (cat: PP-210L, Jena Bioscience GmbH, Germany). The quantity and purity of RNA were assessed by Implen™ NanoPhotometer™ N60 Micro-Volume UV-VIS Spectrophotometer (Implen, Germany), and its integrity was confirmed by absorbance ratios at 260/280 nm. Complementary DNA (cDNA) synthesis was performed using 100 ng of total RNA and the SCRIPT RT-qPCR GreenMaster technique (Cat. No. PCR-520: Jena Bioscince, Germany). Human-specific primers were employed as follows: NF-κB (RELA/p65) forward 5′-GGTTACGGGAGATGTGAAGATG-3′ and reverse 5′-AAGGTGGATGATGGCTAAGTGT-3′. GAPDH was used as the internal reference gene, with forward primer 5′-ACA GCAACAGGGTGGTGGAC-3′ and reverse primer 5′-TTTGAGGGTGCAGCGAACTT-3′ (Bioneer, Daejeon, Republic of Korea). Reverse transcription was performed at 55°C for 15 minutes, followed by pre-denaturation at 95°C for 5 minutes to inactivate reverse transcriptase and activate hot-start DNA polymerase. Amplification was performed with 40 cycles of denaturation (95°C, 15 seconds), annealing (63°C, 20 seconds), and extension (72°C, 30 seconds). Fluorescence signals were collected in the SYBR Green channel during amplification. Relative gene expression levels were calculated using the 2^ΔΔCt method, with normalization to GAPDH expression (Wang et al., 2017). All samples were analyzed in technical duplicate to ensure reproducibility and analytical accuracy.

Primer efficiencies of NF-kappa B and GAPDH were analysed by five-fold serial dilutions of cDNA and were found to range from 95% to 102%, with R^2 > 0.99 (following MIQE guidelines) demonstrating the efficiency of the primers and their quantitative accuracy. Post-PCR melt curve analysis was used to confirm the specificity of amplification, showing a single peak. To exclude contamination and gDNA interference, No-template controls (NTC) and reverse transcriptase-minus controls (No-RT) were added to each run. Intra-assay coefficient of variation (CV) was kept to less than 2% for replication of consistency.

Statistical Analysis

Statistical analyses were conducted using SPSS 28.0 and GraphPad Prism 9.0, with data distribution assessed by Shapiro-Wilk and Kolmogorov-Smirnovtests; continuous variables were summarized as mean ± SD or median (IQR), categorical variables as frequencies and percentages, and group differences evaluated using ANOVA or Kruskal-Wallis tests with appropriate post-hoc analyses and Bonferroni correction to adjust for multiple comparisons, while categorical comparisons used chi-square or Fisher's exact tests. Mutation status was analyzed categorically, NF-κB/p65 expression was calculated using the 2^-ΔΔCt method normalized to GAPDH, and differences were assessed with t-tests or Mann-Whitney U tests. Correlations between molecular and clinical parameters were examined using Pearson's or Spearman's coefficients, with $p < 0.05$ considered statistically significant. Multivariate binary logistic regression analysis was then performed to determine if NF-κB/p65 was an independent predictor of thrombosis and splenomegaly among MPN patients. Potential confounding factors (age, sex, MPN disease subtype [PV, ET, PMF] and JAK2 V617F mutation status) were adjusted. Adjusted Odds Ratios (aOR) with 95% Confidence Intervals (95% CI) were presented (Zaidi, 2024).

Results

Demographic and clinical characteristics

A total of 150 individuals were included in the final analysis, comprising 110 patients with classical MPNs and 40 age- and sex-matched healthy controls. Among the patient cohort, 40 individuals were diagnosed with PV, 40 with ET, and 30 with PMF. The demographic, clinical, hematologic features and the control group are presented in Table 1. The mean age was similar across all groups, including patients with PV, ET, PMF, the general myeloproliferative disorders and the control group, ranging from 55 to 58 years, with no statistically significant difference ($p = 0.45$). The sex distribution also did not differ significantly between the groups, with the proportion of male and female participants being similar ($p = 0.91$).

Splenomegaly was noticed only in MPNs patients and showed considerable variability among disease subtypes ($p < 0.001$). It was most common in patients with primary myelofibrosis (63.3%), followed by patients with polycythemia vera (27.5%) and then patients with essential thrombocythemia (15%). No cases of splenomegaly were recorded in the control group. Previous thrombotic events were reported in 35% of patients with polycythemia vera, 22.5% of those with essential thrombocythemia, and 16.7% of patients with primary myelofibrosis, with a significant difference between groups ($p = 0.02$). Hemorrhagic complications were also limited to the MPN cohort and occurred most often in primary myelofibrosis (20%), compared with polycythemia vera (10%) and essential thrombocythemia (7.5%), resulting in a statistically significant difference ($p = 0.001$).

Hematologic parameters differed significantly among the study groups. Mean hemoglobin levels were highest in PV (18.2 ± 1.6 g/dL), intermediate in ET (13.6 ± 1.4 g/dL), and lowest in PMF (11.4 ± 1.9 g/dL), with overall values not significantly different from those of the control group (14.1 ± 1.2 g/dL; $p = 0.42$). Although hemoglobin levels varied significantly among individual MPN subtypes, no significant difference was seen when the MPN cohort was analyzed as a whole versus controls. This likely reflects the opposing erythroid patterns of polycythemia vera and primary myelofibrosis, which offset each other and increase variability in pooled analyses.

Platelet counts varied considerably ($p < 0.001$), with the highest mean in ET [820 ×10⁹/L (IQR 650–980)], followed by PV [540 ×10⁹/L (IQR 420–690)] and PMF [410 ×10⁹/L (IQR 280–560)], while the control group showed much lower counts [260 ×10⁹/L (IQR 210–310)]. WBC counts also differed considerably between the groups ($p < 0.001$). The highest mean values were observed in PMF (13.2 ± 4.1 ×10⁹/L), followed by PV (11.8 ± 3.6 ×10⁹/L) and ET (9.4 ± 2.8 ×10⁹/L). The control group had the lowest mean WBC count (6.5 ± 1.7 ×10⁹/L).

Table 1: Demographic, Clinical, and Hematological Characteristics of Study Participants

Parameter	PV (n = 40)	ET (n = 40)	PMF (n = 30)	Total MPN (n = 110)	Controls (n = 40)	p-value
Age, years median (IQR)	58 (46-68)	55 (44-66)	57 (47-69)	56 (45-68)	55 (43-65)	0.45
Male, n (%)	22 (55%)	20 (50%)	18 (60%)	60 (53.3%)	21 (52.5%)	0.91

Splenomegaly, n (%)	11 (27.5%)	6 (15%)	19 (63.3%)	36 (32.7%)	0	<0.001
Thrombotic history, n (%)	14 (35%)	9 (22.5%)	5 (16.7%)	28 (25.5%)	0	0.02
Hemorrhagic events, n (%)	4 (10%)	3 (7.5%)	6 (20%)	13 (11.8%)	0	0.001
Hemoglobin (g/dL) Mean ± SD	18.2 ± 1.6	13.6 ± 1.4	11.4 ± 1.9	14.5 ± 3.4	14.1 ± 1.2	<0.42
Platelet count (×10⁹/L) median (IQR)	540 (420-690)	820 (650-980)	410 (280-560)	550 (420-720)	260 (210-310)	<0.001
WBC count (×10⁹/L) Mean ± SD	11.8 ± 3.6	9.4 ± 2.8	13.2 ± 4.1	11.2 ± 3.7	6.5 ± 1.7	<0.001

IQR, Interquartile Range

Frequency and distribution of driver mutations

Table 2 summarizes the distribution of driver mutations in patients with MPNs. The JAK2 V617F mutation was the most common, detected in 68 of 110 patients (61.8%), with the highest prevalence in polycythemia vera (35/40, 87.5%), followed by primary thrombocythemia (20/40, 50.0%) and primary myelofibrosis (13/30, 43.3%). Exon 12 mutations in the JAK2 gene occurred only in polycythemia vera (4/40, 10.0%).

Mutations in exon 9 of the CALR gene were observed in all subtypes, with the highest incidence in ET (12/40, 30.0%), followed by PMF (8/30, 26.7%), and PV (4/40, 10.0%), totaling 24 out of 110 patients (21.8%). Mutations in exon 10 of the MPL gene were less common, found in 6 out of 110 patients (5.5%) across all subtypes. Triple-negative status was observed in all subtypes, most often in PMF (5/30, 16.7%), totaling 10 of 110 patients (9.1%). Patients were classified based on their dominant driver mutation.

Table 2: Distribution of driver mutations in MPN Patients

Mutation Type	PV (n = 40)	ET (n = 40)	PMF (n = 30)	Total (n = 110)
JAK2 V617F	35 (87.5%)	20 (50.0%)	13 (43.3%)	68 (61.8%)
JAK2 exon 12	2 (5.0%)	0	0	2 (1.8%)
CALR exon 9	4 (10.0%)	12 (30.0%)	8 (26.7%)	24 (21.8%)
MPL exon 10	1 (2.5%)	2 (5.0%)	3 (10.0%)	6 (5.5%)
Triple-negative	3 (7.5%)	2 (5.0%)	5 (16.7%)	10 (9.1%)

Correlation between driver mutations and clinical parameters

As shown in Table 3, analysis of clinical parameters stratified by mutational status revealed clear phenotypic distinctions. JAK2-mutated patients were marked by a pronounced erythroid and myeloid burden. Hemoglobin levels were significantly elevated (16.9 ± 2.1 g/dL), and WBC counts were higher than in CALR/MPL and triple-negative groups (12.6 ± 3.8 ×10⁹/L; $p < 0.01$ and $p = 0.02$, respectively). This group also carries the highest risk of thrombosis, with complications observed in 38.2% of cases ($P < 0.01$), confirming the high-risk nature of JAK2 V617F. Clonality: Patients with the CALR/MPL mutation predominantly exhibited a macronuclear phenotype. They reached the highest platelet counts (median 810 ×10⁹/L, IQR 640-970; $p < 0.01$), while hemoglobin and WBC values were comparatively lower. Clinically, splenomegaly was more frequent in this group (54.5%), and thrombotic events were less common (15.2%), highlighting their platelet-driven but less thrombotic profile. Triple-negative patients showed an intermediate hematological pattern. Hemoglobin levels (12.8 ± 1.9 g/dL) and WBC counts (10.1 ± 3.2 ×10⁹/L) were modest, while platelet counts (median 590 ×10⁹/L, IQR 430-720) fell between JAK2 and CALR/MPL groups. Splenomegaly was frequent (50.0%), resembling the CALR/MPL subgroup, whereas thrombotic events occurred in 20% of cases.

Table 3. Association between driver mutations and clinical parameters

Parameter	JAK2-mutated (n = 89)	CALR/MPL-mutated (n = 33)	Triple-negative (n = 10)	p-value

Hemoglobin (g/dL), mean ± SD	16.9 ± 2.1	13.1 ± 1.7	12.8 ± 1.9	<0.01
Platelet count (×10⁹/L), median (IQR)	520 (410-690)	810 (640-970)	590 (430-720)	<0.01
WBC count (×10⁹/L), mean ± SD	12.6 ± 3.8	9.2 ± 2.9	10.1 ± 3.2	0.02
Splenomegaly, n (%)	26 (29.2%)	18 (54.5%)	5 (50%)	0.03
Thrombosis, n (%)	34 (38.2%)	5 (15.2%)	2 (20%)	<0.01

Differential NF-κB/p65 activation according to driver mutation status and disease phenotype

NF-κB/p65 mRNA expression levels across different MPN subtypes, mutation-defined groups, and clinical categories are presented in Figure 1 and Table 4. Healthy control samples showed baseline levels of NF-κB/p65 expression, with a mean increase of 1.00 (IQR 0.85-1.15). In contrast, all subtypes of myeloproliferative disorders showed significantly higher levels of NF-κB/p65 expression comparing with control samples. Among these, patients with PV had a median fold change of 2.7 (IQR 2.1-3.3), those with ET had a median of 2.2 (IQR 1.8-2.9), and patients with PMF exhibited the highest expression levels at 3.6 (IQR 2.9-4.4). All differences were statistically significant ($p < 0.001$).

When patients were stratified by driver mutation status, NF-κB/p65 expression remained significantly elevated across all mutation-defined MPN groups compared with healthy controls. The highest mean gene expression was observed in patients with a mutation in the JAK2 gene [3.2 (IQR 2.6-4.0)], followed by patients with triple-negative results [2.6 (IQR 2.0-3.3)], and then patients with mutations in the CALR/MPL genes [2.4 (IQR 1.9-3.1)]. These results illustrate increased activation of the NF-κB pathway regardless of the underlying molecular trigger.

Additionally, analysis showed, based on clinical characteristics, that patients with a history of thrombotic events had significantly higher expression levels of NF-κB/p65 protein [3.4 (IQR 2.8-4.3)] comparing with healthy individuals ($p < 0.01$). Patients without thrombosis also showed elevated expression levels [2.4 (IQR 1.9-3.1); $p < 0.001$]. Likewise, individuals with splenomegaly shown significantly increased NF-κB/p65 protein expression [3.5 (IQR 2.7-4.2)], whereas those without splenomegaly showed lower, but still significantly elevated levels [2.3 (IQR 1.8-2.9)] compared with healthy individuals ($p < 0.001$ for both comparisons).

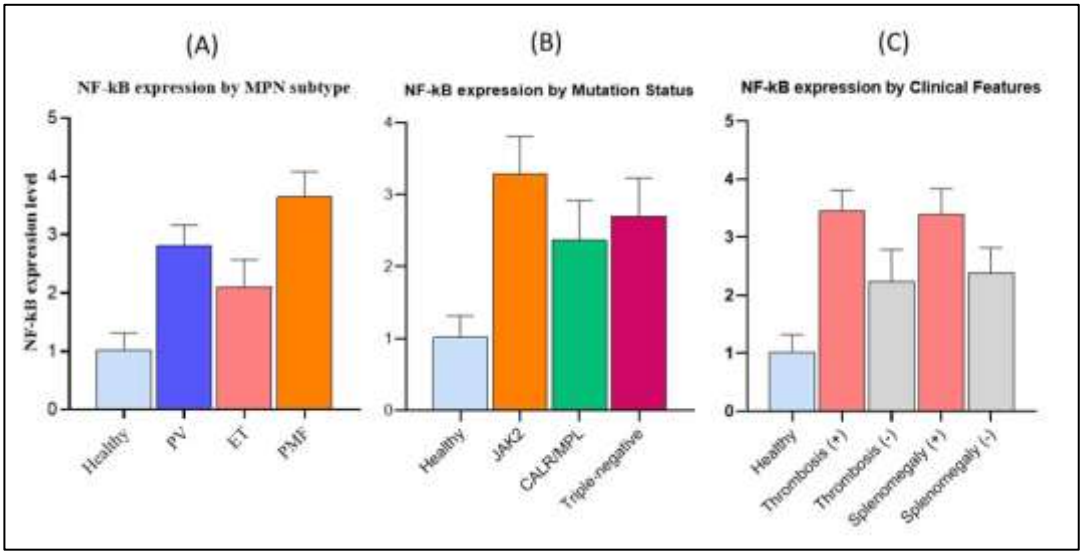

Figure 1: NF-κB/p65 mRNA relative expression ($2^{-}\Delta\Delta Ct$) in classical MPN subtypes and controls.

(A) NF-κB/p65 expression across MPN subtypes [Polycythemia Vera (PV), Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF)] compared to healthy controls. Expression levels were significantly higher in all MPN subtypes, peaking in PMF.

(B) NF-κB/p65 expression stratified by dominant driver mutations (JAK2 V617F/exon 12, CALR/MPL, and Triple-Negative). JAK2-mutated cases exhibited the highest expression compared to other mutation profiles.
(C) NF-κB/p65 expression according to clinical manifestations, showing significantly elevated fold-changes in patients with a history of thrombosis and splenomegaly.
Data are presented as median values with interquartile ranges (IQR; represented by boxes) and min-max range (whiskers). Horizontal dotted lines represent baseline expression in healthy controls (mean = 1.00). Statistical significance between groups was determined using the Mann-Whitney U test / Kruskal-Wallis test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. healthy controls or indicated reference groups.

Table 4. NF-κB/p65 mRNA expression in MPN Patients according to subtype, mutation status, and clinical features

Category	Subgroup	NF-κB/p65 Fold Change ($2^{-}\Delta\Delta Ct$), median (IQR)	p-value
Controls	Healthy (n = 40)	1.00 (0.85-1.15)	--
MPN Subtypes	PV (n = 40)	2.7 (2.1-3.3)	<0.001 vs controls
	ET (n = 40)	2.2 (1.8-2.9)	<0.001 vs controls
	PMF (n = 30)	3.6 (2.9-4.4)	<0.001 vs controls
Mutation Status	JAK2-mutated (n = 70)	3.2 (2.6-4.0)	0.002 vs CALR/MPL
	CALR/MPL-mutated (n = 33)	2.4 (1.9-3.1)	Reference
	Triple-negative (n = 10)	2.6 (2.0-3.3)	0.01 vs CALR/MPL
Clinical Features	Thrombosis present	3.4 (2.8-4.3)	0.01 vs no thrombosis
	No thrombosis	2.4 (1.9-3.1)	Reference
	Splenomegaly present	3.5 (2.7-4.2)	0.01 vs no Splenomegaly
	No splenomegaly	2.3 (1.8-2.9)	Reference

Each patient was classified according to the dominant driver mutation. P-values for MPN subtypes represent comparisons with healthy controls. For mutation status and clinical features, p-values represent comparisons with the indicated reference subgroup

Independent Association of NF-κB/p65 Expression with Clinical Features
To adjust for potential baseline confounders (such as age, sex, MPN subtypes, and JAK2 V617F mutation status), a multivariate binary logistic regression model was constructed (Table 5). High NF-κB/p65 expression remained an independent predictor for thrombotic events (Adjusted OR = 2.45, 95% CI: 1.35-4.42, p = 0.003). Similarly, elevated NF-κB/p65 expression was independently associated with the presence of splenomegaly (Adjusted OR = 3.12, 95% CI: 1.74-5.58, $p < 0.001$) after adjusting for all covariates.

Table 5. Multivariate Binary Logistic Regression Analysis for Predictors of Thrombosis and Splenomegaly in MPN Patients

Clinical Outcome / Variables	Adjusted Odds Ratio	95% CI	p-value
Thrombotic Events			
NF-κB/p65 expression	2.45	1.35 - 4.42	0.003

Age	1.02	0.98 - 1.06	0.34
JAK2 V617F status	2.10	1.05 - 4.20	0.036
MPN Subtype (PV vs ET/PMF)	1.85	0.88 - 3.88	0.10
Splenomegaly			
NF-κB/p65 expression	3.12	1.74 - 5.58	<0.001
PMF Subtype	4.80	2.10 - 10.95	<0.001
JAK2 V617F status	1.40	0.65 - 3.01	0.39

Discussion

The findings of this study provide a definitive link between NF-κB dysregulation and the clinical landscape of Philadelphia chromosome-negative MPNs. By synthesizing molecular, transcriptional, and clinical insights from a unique Iraqi cohort, our data reposition NF-κB not merely as a bystander, but as the mechanistic bridge where oncogenic mutations and systemic inflammation collide. This convergence suggests that the NF-κB pathway is the master engineer of the disease phenotype, determining both biological severity and clinical manifestations (Fisher et al., 2021; Kleppe et al., 2015). Previous studies have identified an inflammatory pathway in MPNs, but this study is the first to directly link transcriptional profiling of NF-κB/p65 to driver mutation status and hard clinical endpoints in a well-defined, human cohort. Importantly, our results showed that the activation of NF-κB is not only a secondary reactive marker, but also an independent clinical driver, which is a more accurate risk stratification than standard mutational and hematological profile (Fisher et al., 2021).

In this study, the groups were matched for age and sex, with no statistically significant differences ($p = 0.45$ and $p = 0.91$, respectively). This close matching strengthens the findings, ensuring that the observed hematological and clinical differences are attributable to the MPN itself, rather than to demographic variation. The mean age of the participants ranged from 55 to 58 years, which roughly corresponds to the peak global prevalence of myeloproliferative disorders (Barbui et al., 2018). Although this age is slightly younger than the age typically reported in large Western registries (67–68 years), it aligns well with recent studies from the Middle East and Asia, where MPNs are commonly diagnosed during the sixth decade of life (Najim and Dlawar, 2022; Zolkafli et al., 2025). These comparisons with populations in the West, however, should be only made with caution. The age at presentation may be younger due to regional differences in demographic structure, as well as different structures of the healthcare system, patterns of clinical referral and screening practices. It is therefore important to increase the size of regional epidemiological registries to confirm these trends and to distinguish real biological variation from ascertainment factors related to the health system. This age consistency is crucial, as it reduces the potential confounding effects of age-related changes in bone marrow function or clonal hematopoiesis, thus reinforcing the notion that the observed differences in hematopoietic cell proliferation and vascular complications are indeed related to the disease.

One of the most significant findings was the marked variation in erythropoiesis expansion between subtypes, particularly the pronounced erythropoiesis in (PV) and the cytopenic tendencies observed in (PMF). The mean hemoglobin level in PV was 18.2 ± 1.6 g/dL; however, when comparing the MPN group with the control group, the difference was not statistically significant ($p = 0.42$). This apparent neutrality reflects the balance in erythropoiesis characteristics between PV and PMF, where one cancels out the other in pooled analyses. These findings underscore the importance of the 2022 WHO and the International Consensus Classification (ICC) criteria, which emphasize specific diagnostic and risk thresholds for each subtype (Arber et al., 2022; Mao et al., 2025). Splenomegaly was significantly more common in primary myelofibrosis (63.3%) than polycythemia vera or essential thrombocythemia ($p < 0.001$), highlighting the shift from myelogenous to extra myelogenous hematopoiesis. In addition to its diagnostic value, splenomegaly reflects the underlying disease; blood retention and splenomegaly likely contribute to the marked leukocytosis observed in PMF (13.2 \pm 4.1 \times 10^9/L). Elevated WBC counts are now a widely recognized independent predictor of decreased survival and increased risk of transformation, contributing to the inflammatory cytokine environment characteristic of advanced

myeloproliferative disorders. Vascular complications remain the primary source of morbidity, with marked differences between subtypes. The thrombotic rate in PV (35%) exceeds that reported in several European registries (15-20%) (Mao et al., 2025), suggesting either a more aggressive thrombotic phenotype or a later disease onset. In contrast, the 20% bleeding rate in PMF ($p = 0.001$) reflects specific platelet dysfunction and acquired von Willebrand syndrome, which often accompanies advanced fibrosis (Papageorgiou et al., 2022).

The distribution of driving mutations in our cohort largely reflects the 2022 WHO and ICC molecular hierarchy, while revealing subtle differences in triple-negative and atypical phenotypes. In polycythemia vera, JAK2 V617F mutations and exon 12 mutations were found in 87.5% and 5.0% of cases, respectively, resulting in an overall JAK2 positivity rate of 92.5%, confirming JAK2's role as a central molecular marker for RBC expansion. Notably, 10% of PV cases carried CALR mutations, suggesting the possibility of "masked" polycythemia vera or phenotypic overlap within Philadelphia chromosome-negative myeloproliferative disorders (Tefferi and Barbui, 2023). In both essential thrombocythemia (ET) and primary myelofibrosis (PMF), mutation patterns were more varied. The JAK2 V617F mutation remained the most prevalent (50.0% in ET and 43.3% in PMF), followed by exon 9 mutations in the CALR gene (30.0% and 26.7% respectively). These patterns are consistent with studies indicating that CALR-mutated thrombocythemia is associated with higher platelet counts and a lower risk of thrombosis compared to JAK2-mutated thrombocythemia (Park et al., 2015). The prevalence of triple-negative thrombocythemia was highest in PMF (16.7%) compared to ET (5.0%) and PV (7.5%), consistent with its association with severe disease and an increased risk of leukemia progression (Tefferi and Barbui., 2023). These results underscore the utility of secondary molecular screening via next-generation gene sequencing for atypical mutations (such as ASXL1 and SRSF2) to improve predictive classification according to ICC guidelines.

The results illustrate that the driving mutational status remains the primary determinant of both clinical symptoms and hematological characteristics in Philadelphia negative myeloproliferative disorders. The JAK2 mutation group showed significantly elevated in level of hemoglobin (16.9 ± 2.1 g/dL) and WBC count, reflecting the typical myeloproliferative activity of PV, where the JAK2 V617F mutation induces persistent signaling and promotes RBC proliferation (Arber et al., 2022). The high incidence of thrombosis (38.2%) in this group strongly supports the established association between JAK2 positivity, leukocytosis, and a predisposition to thrombosis (Cuthbert and Stein, 2019). In contrast, patients with CALR or MPL mutations exhibited a distinct megakaryocyte pattern, with a markedly elevated mean platelet count (810×10^9/L). This "essential thrombocytosis-like" phenotype is typically associated with lower hemoglobin levels and a reduced risk of thrombosis compared to JAK2-mutant patients, consistent with recent longitudinal studies indicating that CALR mutations lead to a more benign disease course despite overt thrombocytosis (Tefferi et al., 2014). Notably, splenomegaly was more prevalent in CALR/MPL patients (54.5%) and triple-negative patients (50.0%) compared to the JAK2 group (29.2%), which may reflect a higher proportion of PMF or pre-PMF cases in these genotypes, as defined by the updated ICC criteria (Maggioni and Della Porta., 2023).

One of the most notable findings of this study is the widespread activation of the NF-κB/p65 signaling pathway across all types of myeloproliferative disorders. While healthy individuals showed only baseline activity, patients with MPNs had markedly higher NF-κB/p65 mRNA levels, with the strongest increase seen in primary myelofibrosis (PMF), a 3.6-fold rise ($p < 0.001$). This gradient of expression, PMF > PV > ET, mirrors the escalating severity of inflammation that defines these diseases. Our results strengthen the growing view that NF-κB is not just a bystander but a central link between the genetic mutations driving MPNs and the inflammatory environment that fuels bone marrow scarring (Kleppe et al., 2015).

When analyzed by mutation type, NF-κB activation was highest in patients with JAK2 mutations (3.2-fold), surpassing those with CALR/MPL mutations and even triple-negative cases. This suggests that the JAK2 V617F clone is a particularly powerful trigger of NF-κB signaling, likely through cross-talk with the JAK-STAT pathway. Yet, the fact that all mutation groups, including triple-negative patients

(2.6-fold), showed significant activation underscores NF-κB as a potential unifying hallmark of MPN biology. This may explain the high proportion of triple-negative primary myelofibrosis in our cohort (16.7%), a subgroup known for poor survival outcomes. (Rumi and Cazzola, 2017).

Clinically, elevated NF-κB/p65 levels were strongly linked to worse disease features. Patients with a history of thrombosis had a 3.4-fold increase, while those with splenomegaly showed a 3.5-fold rise ($p < 0.001$). The association with thrombosis ($p < 0.01$) is of particular interest: NF-κB given established regulatory role in pro-inflammatory and prothrombotic genes, and its elevated expression may create

a "predisposing" environment for serious vascular occlusions (Papageorgiou et al., 2022). A direct causative mechanism could only be proven if functional inhibition or downstream assays could be performed, but this is not possible. In the same fashion, the increased levels of NF-κB in patients with splenomegaly correlate with the known function of NF-κB in microenvironmental inflammation, but whether increased splenic NF-κB activity directly contributes to increased disease bulk or is a secondary systemic response is not fully explained.

Crucially, our multivariate regression analysis shows that the link between high levels of NF-κB/p65 mRNA expression and poor clinical outcome (thrombosis and splenomegaly) does not depend on underlying JAK2 signaling or on specific disease phenotypes. Up-regulation of NF-κB/p65 was also an independent predictor of thrombosis ($p = 0.003$) and splenomegaly ($p < 0.001$), even following adjustment for age, sex, disease subtype and mutational status. This is evidence that inflammatory pathway dysregulation through NF-κB is an independent pathway to clinical complications in classical MPNs (Ferrer-Marín et al., 2021). From a practical standpoint, the independent association of NF-κB/p65 with thrombosis and splenomegaly offers a tangible clinical advantage: it highlights NF-κB expression as a valuable surrogate biomarker to identify 'high-inflammatory phenotype' patients who remain at elevated vascular risk despite adequate cytoreductive therapy. In addition, may offers a biological justification for the use of combinations of JAK inhibitors and anti-inflammatory (or NF-κB targeting) agents to achieve therapeutic benefits in overcoming the remaining inflammatory disease burden.

Ultimately, our findings define distinct clinical and molecular pathways for PV, ET, and PMF. Our results highlight the NF-κB/p65 axis as a central mediator of myeloproliferative patterns, as it is associated with JAK2 mutation status, vascular risk and disease severity. These data support a shift toward personalized, risk-adapted treatment strategies that integrate both hematological clinical criteria and inflammatory genetic triggers to improve long-term outcomes (Mao et al., 2025).

This study's strength lies in its integrated design, combining clinical, hematologic, molecular, and signaling data within a well-defined cohort. Age- and sex-matched controls allowed us to isolate disease-specific effects of MPNs. By including all three classical BCR-ABL1-negative subtypes (PV, ET, PMF), we directly compared their clinical and molecular features. A major focus was the NF-κB/p65 pathway. Unlike studies limited to driver mutations, we examined the inflammatory environment shaping disease progression. Linking NF-κB activity to mutation status and complications such as thrombosis and splenomegaly revealed a shared inflammatory axis across MPNs. This highlights NF-κB not only as a marker of disease activity, but as a potential candidate for future therapeutic exploration, supporting the rationale for investigating strategies beyond standard JAK inhibition.

Despite its strengths, this study has several limitations. It was a single-center, cross-sectional analysis, and while the overall sample size was adequate, smaller subgroups, like triple-negative PMF or MPL-mutated cases, limit some genotype–phenotype conclusions. Moreover, there is a lack of functional experiments to validate the suggested mechanistic interactions between NF-κB and certain mutations and clinical characteristics, which are now only speculative. Our molecular assessment focused only on the three main driver mutations (JAK2, CALR, MPL), without next-generation sequencing to detect additional high-risk mutations such as ASXL1, SRSF2, or EZH2, which may explain the elevated NF-κB activation in triple-negative cases. Finally, the retrospective thrombotic data prevent us from proving causality, making prospective, longitudinal studies necessary to see if NF-κB levels can predict vascular complications or disease progression. Overall, these limitations highlight the need for larger, multi-center studies combining comprehensive genomics with long-term follow-up to clarify NF-κB's role in MPNs and guide personalized therapies. Furthermore, since our results were obtained from observation, we are not yet able to validate the function nor perform drug-response assays, our translation conclusions about NF-κB as a therapeutic target are hypothesis-generating. Also, mechanistic studies and translational trials are required before NF-κB targeting can be integrated into clinical practice

Conclusion

This study identifies NF-κB/p65 signaling axis as an independent clinical and biological marker in BCR-ABL1-negative MPNs for an Iraqi cohort. NF-κB activation is correlated with the severity of disease (PMF > PV > ET) and is a common feature in all mutational profiles, even in triple-negative cases. Significantly, high expression of NF-κB is a standalone predictor of thrombotic risk and splenomegaly. While the younger age at presentation underscores distinct regional characteristics, these

findings highlight the potential of targeting the NF-κB pathway alongside standard therapies to mitigate inflammatory and vascular complications in MPN patients.

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Conflicts of Interest:
Authors declare that they have no conflict of interest for this study.

Reference

1. Al-Dayyeni, A. M., Al-Gailani, B. T., Mahdi, M. G. & Khazeem, M. M. (2023). The prevalence of JAK2, CALR, and MPL mutations in BCR-ABL1 rearrangement-negative Iraqi patients. Journal of Population Therapeutics and Clinical Pharmacology. 30(3):146-152. doi:10.47750/jptcp.2023.30.03.046.
2. Arber, D. A., Orazi, A., Hasserjian, R. P., Borowitz, M. J., Calvo, K. R., Kvasnicka, H. M., et al. (2022). International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 140:1200-28. doi: 10.1182/blood.2022015850.
3. Baumeister, J., Maié, T., Chatain, N., Gan, L., Weinbergerova, B., de Toledo, M. A. S., Eschweiler, J., Maurer, A., Mayer, J., Kubesova, B., Racil, Z., Schuppert, A., Costa, I., Koschmieder, S., Brümmendorf, T. H., & Gezer, D. (2021). Early and late stage MPN patients show distinct gene expression profiles in $CD34^+$ cells. Annals of Hematology, 100(12), 2943-2956. https://doi.org/10.1007/s00277-021-04615-8.
4. Barbui, T., Tefferi, A., Vannucchi, A. M., Passamonti, F., Silver, R. T., Hoffman, R., Verstovsek, S., Mesa, R., Kiladjian, J. J., Hehlmann, R., Reiter, A., Cervantes, F., Harrison, C., Mc Mullin, M. F., Hasselbalch, H. C., Koschmieder, S., Marchetti, M., Bacigalupo, A., Finazzi, G., Kroeger, N., Griesshammer, M., Birgegard, G., & Barosi, G. (2018). Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European Leukemia Net. Leukemia, 32(5): 1057-1069. https://doi.org/10.1038/s41375-018-0077-1
5. Collins, P. E., Mitxitorena, I. & Carmody, R. J. (2016). The Ubiquitination of NF-κB Subunits in the Control of Transcription. Cells, 5(2), 23. DOI: 10.3390/cells5020023
6. Costa, A. & Breccia, M. (2025). Challenges in the Evolving Role of Calreticulin as a Promising Target for Precision Medicine in Myeloproliferative Neoplasms. Cancers, 17(21), 3397. https://doi.org/10.3390/cancers17213397
7. Cuthbert, D. & Stein, B. L. (2019). Polycythemia Vera-Associated Complications: Pathogenesis, Clinical Manifestations, And Effects on Outcomes. Journal of Blood Medicine. 18(10):359-371. doi: 10.2147/JBM.S189922. PMID: 31695542; PMCID: PMC6805785.
8. Fisher, D. A. C., Fowles, J. S., Zhou, A. & Oh, S. T. (2021). Inflammatory Pathophysiology as a Contributor to Myeloproliferative Neoplasms. Frontiers in Immunology. 1;12: 683401. doi: 10.3389/fimmu.2021.683401. PMID: 34140953; PMCID: PMC8204249.
9. Ferrer-Marín, F., Cuenca-Zamora, E. J., Guijarro-Carrillo, P. J. and Teruel-Montoya, R. (2021). Emerging Role of Neutrophils in the Thrombosis of Chronic Myeloproliferative Neoplasms. International Journal of Molecular Sciences. 22, 1143. https://doi.org/10.3390/ijms22031143.
10.Gerds, A. T., Gotlib, J., Ali, H., Bose, P., Dunbar, A., Elshoury, A., George, T. I., Gundabolu, K., Hexner, E., Hobbs, G. S., et al. (2022). Myeloproliferative Neoplasms, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network, 20(9): 1033-1062.
11.Grinfeld, J., Nangalia, J. & Green, A. R. (2017). Molecular determinants of pathogenesis and clinical phenotype in myeloproliferative neoplasms. Haematologica. 102(1):7-17. doi: 10.3324/haematol.2014.113845.
12.Guha, P., Das, A., Dutta, S. & Chaudhuri, T. K. (2018). A rapid and efficient DNA extraction protocol from fresh and frozen human blood samples. The Journal of Clinical Laboratory Analysis. 32(1): e22181. doi: 10.1002/jcla.22181. Epub 2017 Feb 23. PMID: 28233351; PMCID: PMC6817243.
13.Hermouet, S. (2023). Mutations, inflammation and phenotype of myeloproliferative neoplasms. Front Oncol. 22(13):1196817. doi: 10.3389/fonc.2023.1196817.

14.Hoffmann, A., Cheng G. & Baltimore, D. (2025). NF-κB: master regulator of cellular responses in health and disease. Immunity & Inflammation, 1(1): 2. DOI: 10.1007/s44466-025-00014-0.
15.Kagoya, Y., Yoshimi, A., Tsuruta-Kishino, T., Arai, S., Satoh, T., Akira, S., & Kurokawa, M. (2014). JAK2V617F+ myeloproliferative neoplasm clones evoke paracrine DNA damage to adjacent normal cells through secretion of lipocalin-2. Blood, 124(19): 2996-3006.
https://doi.org/10.1182/blood-2014-04-570572.
16.Kleppe, M., Kwak, M., Koppikar, P., Riester, M., Keller, M., Bastian, L., Hricik, T., Bhagwat, N., McKenney, A. S., Papalexi, E., Abdel-Wahab, O., Rampal, R., Marubayashi, S., Chen, J. J., Romanet, V., Fridman, J. S., Bromberg, J., Teruya-Feldstein, J., Murakami, M., Radimerski, T., Michor, F., Fan, R., & Levine, R. L. (2015). JAK-STAT pathway activation in malignant and nonmalignant cells contributes to MPN pathogenesis and therapeutic response. Cancer Discovery, 5(3), 316-331.
DOI: 10.1158/2159-8290.CD-14-0736.
17.Lee, K. & Tripathi, A. (2020). Parallel DNA Extraction from Whole Blood for Rapid Sample Generation in Genetic Epidemiological Studies. Frontiers in Genetics. 29(11):374.
doi: 10.3389/fgene.2020.00374. Luque Paz, D., Kralovics, R. & Skoda, R. C. (2023). Genetic basis and molecular profiling in myeloproliferative neoplasms. Blood. 20;141(16):1909-1921.
doi: 10.1182/blood.2022017578.
18.Maggioni, G. & Della Porta, M. G. (2023). Molecular landscape of myelodysplastic neoplasms in disease classification and prognostication. Current Opinion in Hematology. 1;30(2): 30-37.
doi: 10.1097/MOH.0000000000000752.
19.Mao, H., Zhao, X. & Sun, S. C. (2025). NF-κB in inflammation and cancer. Cellular & Molecular Immunology. 22(8):811-839. doi: 10.1038/s41423-025-01310-w.
20.Morsia, E., Torre, E., Poloni, A., Olivieri, A. & Rupoli S. (2022). Molecular Pathogenesis of Myeloproliferative Neoplasms: From Molecular Landscape to Therapeutic Implications. International Journal of Molecular Sciences. 23(9):4573. https://doi.org/10.3390/ijms23094573.
21.Najim, M. B. & Dlawar, S. (2022). The Impact of JAK2 V617F, CALR, and MPL Mutations as Molecular Diagnostic Markers of Myeloproliferative Neoplasms in Kurdish Patients. A Single-center Experience. Cellular and Molecular Biology, 68(8): 202-209.
https://doi.org/10.14715/cmb/2022.68.8.34.
22.Nguyen, L. T., Pollock, C. A. & Saad, S. (2024). Extraction of high quality and high yield RNA from frozen EDTA blood. International Journal of Scientific Reports. 14:8628. doi:10.1038/s41598-024-58576-9.
23.Pan, B., Shi, K., Li, Y., Wang X., Zhang, Q. & Li, L. (2026). JAK-STAT pathway in hematological malignancies: Achievements, challenges and future perspectives. Biomedicine and pharmacotherapy. 6(195):118959. doi: 10.1016/j.biopha.2025.118959.
24.Papageorgiou, L., Elalamy, I., Vandreden, P. & Gerotziafas, G. T. (2022). Thrombotic and Hemorrhagic Issues Associated with Myeloproliferative Neoplasms. Clinical and Applied Thrombosis/Hemostasis, 28: 1-11. DOI: 10.1177/10760296221097969
25.Park, S. H., Kim, S. Y., Lee, S. M., Yi, J., Kim, I. S., Kim, H. H., Chang, C. L., Lee, E. Y., Song, M. K., Shin, H. J., & Chung, J. S. (2015). Incidence, clinical features, and prognostic impact of CALR exon 9 mutations in essential thrombocythemia and primary myelofibrosis: an experience of a single tertiary hospital in Korea. Annals of Laboratory Medicine, 35(2): 233-237.
https://doi.org/10.3343/alm.2015.35.2.233
26.Rumi, E. & Cazzola, M. (2017). Diagnosis, risk stratification, and response evaluation in classical myeloproliferative neoplasms. Blood. 9;129(6): 680-692. doi: 10.1182/blood-2016-10-695957. Epub 2016 Dec 27.
27.Stivala, S. & Meyer, S. C. (2021). Recent Advances in Molecular Diagnostics and Targeted Therapy of Myeloproliferative Neoplasms. Cancers (Basel), 13(20): 5035.
https://doi.org/10.3390/cancers13205035.
28.Soyfer, E. M & Fleischman, A. G. (2023). Myeloproliferative neoplasms-blurring the lines between cancer and chronic inflammatory disorder. Frontiers in Oncology, 13:1208089.
DOI: 10.3389/fonc.2023.1208089.
29.Sullivan, J. Y. & Fleischman, A. G. (2024). Relating NF-κB regulation to MPN pathogenesis. Blood, 143(23): 2345-2347. DOI: 10.1182/blood.2024024451.
30.Tefferi, A. & Barbui, T. (2020). Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. The American Journal of Hematology. 95(12):1599-1613. doi: 10.1002/ajh.26008.

31.Tefferi, A., Guglielmelli, P., Larson, D. R., Finke, C., Wassie, E. A., Pieri, L., Gangat, N., Fjerza, R., Belachew, A. A., Lasho, T. L., Ketterling, R. P., Hanson C. A., Rambaldi, A., Finazzi, G., Thiele, J., Barbui, T., Pardanani, A., & Vannucchi, A. M. (2014). Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood, 124(16): 2507-2513. doi: 10.1182/blood-2014-05-579136.
32.Tefferi A, Pardanani A. Myeloproliferative Neoplasms: A Contemporary Review. JAMA Oncology. 2015 Apr;1(1):97-105. doi: 10.1001/jamaoncol.2015.89..
33.Tefferi, A. & Barbui, T. (2023). Polycythemia vera: 2024 update on diagnosis, risk-stratification, and management. American Journal of Hematology, 98(9): 1465-1487. DOI: 10.1002/ajh.27002.
34.Wang, X., Ma, N., Sun, Q., Huang, C., Liu, Y. & Luo, X. (2017). Elevated NF-κB signaling in Asherman syndrome patients and animal models. Oncotarget. 28;8(9):15399-15406. doi: 10.18632/oncotarget.14853.
35.Zeng, Y., Tang, X., Chen, J., Kang, X. & Bai, D. (2024). Optimizing total RNA extraction method for human and mice samples. Peer Journal. 26(12):e18072. doi: 10.7717/peerj.18072.
36.Zolkafli, N. B., Cheong, C. S., Anuar, N. A., Nadarajan, V. S., Bee, P. C., Chin, E. F. M., Khairullah, S., Liong, C. C., Zamri, M. Y., & Gan, G. G. (2025). The Clinical Characteristics and Outcomes of JAK2/CALR/MPL Mutation Related Myeloproliferative Neoplasms - A Single Centre Study from Malaysia. IIUM Medical Journal Malaysia, 24(1): 83-91.
https://doi.org/10.31436/imjm.v24i1.2560
37.Zaidi, A. U. (2024). A Guide for Statistical Tests and Interpretations with SPSS. 1st Edition. published by Routledge (an imprint of Taylor & Francis). New York.
https://doi.org/10.4324/9781003215691.