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# Prevalence of Factor V Leiden Mutation in Iraqi Patients with Essential Thrombocythemia and its Association with MPN Driver Mutations: A Retrospective Cross-Sectional Study

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## Abstract

Essential thrombocythemia (ET) is a clonal myeloproliferative neoplasm caused by somatic mutations in JAK2, CALR, or MPL in around 80% of patients, with the remainder classified as triple-negative ET (TN-ET). Factor V Leiden (FVL) is the predominant genetic thrombophilia, and the prevalence of FVL among Iraqi ET patients remain ambiguous. This study hypothesised that the FVL may be an overlooked contributor to thrombotic risk in TN-ET.

To determine the prevalence of FVL in Iraqi ET patients, to define its coexistence with MPN driver mutations, and to examine its association with hematological markers.

A retrospective cross-sectional study was performed at Medical City Hospital in Baghdad from November 2025 to February 2026. Fifty ET patients were assessed for MPN driver mutations using the TRUPCR® MPN Panel and for FVL by PCR and Sanger sequencing. Fisher's exact test and logistic regression were employed. A post-hoc power analysis was performed.

FVL was identified in 21 patients (42.0%), MPN driver mutations in 8 patients (16.0%), and 42 patients (84.0%) were triple-negative. FVL was significantly associated with MPN positive (OR 14.00, 95% CI 1.52–63.36; p = 0.007) and remained significant after adjusting for age and sex (OR 13.56, 95% CI 1.46–126.37; p = 0.022). Fourteen individuals with TN-ET tested positive for FVL, constituting 33.3% of the TN-ET subgroup. No significant hematological differences were observed among the mutational categories. The post hoc power was 0.82.

This Iraqi analysis study reveals a notable prevalence of FVL and a strong independent correlation between FVL and MPN. Our hypothesis that genetic APC resistance may constitute the primary prothrombotic mechanism in these patients is corroborated by the FVL-positive TN-ET subgroup. Routine FVL testing is recommended in Iraqi ET practice regardless of driver mutation status.

**Keywords:** Essential Thrombocythemia, Factor V Leiden, Triple-negative ET, Thrombophilia.

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## Introduction

Essential thrombocythemia (ET) is a chronic clonal myeloproliferative neoplasm, defined by persistent thrombocytosis, which occurs as a result of autonomous proliferation of the megakaryocytic lineage, and which is clinically linked with a high risk of both arterial and venous thrombotic complications (1,2).

Around 80% of patients, molecular definition of the disease was one of three mutually exclusive driver mutations, all of which lead to the constitutive activation of *JAK-STAT* signaling pathway: *JAK2 V617F*, *CALR exon 9* and *MPL exon 10* (1-5). The other 10–20% of patients who meet all the clinical and morphological criteria for ET but are negative for all three canonical mutations are classified as triple-negative ET (TN-ET), and are a biologically heterogeneous group without a clear pathogenetic basis, with diagnosis being based only on the absence of reactive thrombocytosis and other myeloid neoplasms (6-9).

Patients with unprovoked persistent thrombocytosis in haematological practice often present with a situation that is clinically challenging, and the three driver mutations of MPNs are only a small portion of the diagnostic puzzle. Thrombocytosis and hypercoagulability often coexist, are well recognized and may be due to inherited thrombophilia disorders, such as the Factor V Leiden (FVL) mutation, which can complicate, mimic, or be part of the clinical picture of ET (10). FVL is a point mutation (*c.1691G>A; p.Arg506Gln*) that makes Factor Va resistant to APC inactivation and is found in heterozygous carriers with a lifelong increased risk of VTE (3-5-fold) (10). Critically, the thrombotic risk of FVL with ET — especially with JAK2 mutant ET — is more than additive — it is significantly higher than the risks of either alone (11,12). In a clinic, however, FVL testing is not routinely done in all patients in whom ET is suspected, especially in resource-poor countries, where the molecular analysis may be limited to the three MPN driver mutations. This leaves a patient group who are at risk of being misdiagnosed with ET, who also have thrombocytosis and who are negative for all three driver mutations and who are considered triple-negative, but for whom an underlying inherited thrombophilia, such as FVL, could be the main or an important underlying factor in their haematological and thrombotic phenotype. The role of FVL as a true co-driver of disease in TN-ET, an independent explanatory factor for the thrombotic tendency in this subgroup or a coincidental finding is an unanswered and clinically relevant question (6,10,12).

No data have been published regarding the co-occurrence of the Factor V Leiden (FVL) mutation and essential thrombocythemia (ET)—particularly the calreticulin-negative subtype (TN-ET)—in Iraq or the Middle East region more broadly, despite global advances in the molecular characterization of myeloproliferative neoplasms (MPNs). The genetic makeup of the Iraqi population is characterized by distinct features, such as high rates of consanguinity; this may contribute to a higher prevalence of hereditary thrombophilia and a molecular spectrum of MPNs that differs from that observed in Western reference populations (13, 14).

This study was designed to evaluate the prevalence of the Factor V Leiden (FVL) mutation among patients suspected of having essential thrombocythemia (ET) at a specialized hematology center in Baghdad, Iraq; to determine the

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frequency of the association between FVL, driver mutations for myeloproliferative neoplasms (MPN), and triple-negative status; and to examine hematological characteristics across different mutation subgroups. The overarching goal was to determine whether FVL acts as an under-recognized prothrombotic factor in Iraqi patients suspected of having ET, particularly within the triple-negative subgroup.

## Material and Methods:

### *Study design*

This study was designed as a retrospective cross-sectional study, involving the examination of patient data and molecular testing records for individuals referred for the detection of the causative mutation (driver mutation) associated with essential thrombocythemia at the Hematology Center of the Medical City Hospital in Baghdad, Iraq, between November 28, 2025, and February 28, 2026.

### *Study Population*

50 patients with a confirmed diagnosis of ET were consecutively recruited during the study period from haematology services at the study centre. Patients were all diagnosed by the World Health Organisation (WHO) 5th Edition criteria for platelet counts of >450×10⁹/L, bone marrow megakaryocytic hyperplasia, and the exclusion of other myeloid neoplasms (1,2). Patients were included if they had a definitive diagnosis of ET, if they could provide an adequate peripheral blood sample, and if they provided informed, written consent. Thrombocytosis, due to a known reactive cause (such as iron deficiency anemia, an active inflammatory or infective state, or post-splenectomy status), was excluded, as was a concurrent diagnosis of another primary myeloid neoplasm.

### *Sample Collection and DNA Extraction*

Whole blood is the main biological sample used in all molecular analyses, and was collected from each patient who enrolled in the study by venipuncture from the peripheral veins, in vacutainer tubes containing EDTA, under standard aseptic precautions. Peripheral blood leucocytes were used for genomic DNA extraction with the Invitrogen™ PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA; Cat. This is done according to the manufacturer's instructions (No. K182001). All specimens with an absorbance ratio of A260/A280 of 1.8 to 2.0 were advanced to molecular analysis, and only those with a higher ratio were discarded. Those with an A260/A280 ratio of 1.8 to 2.0 were advanced to molecular analysis; those with a higher ratio were discarded, and DNA integrity was confirmed spectrophotometrically.

### *MPN Driver Mutation Analysis*

The TRUPCR® MPN Panel Kit (3B BlackBio Biotech India Ltd., Bhopal, India; Product Nos. 5000, 5001, 5002) was used for the detection of the three canonical ET driver mutations (JAK2 V617F (exon 14), CALR exon 9 frameshift variants, and MPL exon 10 W515L/K substitutions). The data were obtained using the 3B1303/3B1304 CE-IVD certified Version 2.1 Real-Time PCR System (Daan Gene Co., Ltd, Guangzhou, China) as described previously (3). The manufacturer states that the analytical range of the TRUPCR® MPN Panel Kit is from 10 copies/reaction to 100 copies/reaction for each mutation, and the level of detection is 1% mutant allele frequency. Validity of the assay was confirmed by positive controls (plasmid DNA containing known mutations), negative controls (wild-type human genomic DNA) and no-template controls.

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Patients were determined to be mutation-positive if one of the three canonical mutations was present, and triple-negative if all three mutations were not present on the full panel. Note that confirmatory Sanger sequencing was not used for MPN driver mutations, only the real-time PCR panel results were used for classification.

### *Factor V Leiden Mutation Detection*

The detection of the FVL mutation (F5 c.1691G>A; rs6025) was done using conventional polymerase chain reaction (PCR) and bidirectional Sanger sequencing. The sequences of the oligonucleotide primers were designed according to the sequences described by Guzmán et al. (2015) (15) for the F5 gene, which contains the activated protein C cleavage site at Arg506 in exon 10.

- Forward (FV10aF): 5′-TGCAAATGAAAACAATTTTGAA-3′
- Reverse (FV10aR): 5′-CTATTAGCCCAGAGGCGATG-3′
- Amplicon size: 202 bp (expected)
- Annealing temperature: 58°C

The PCR reaction mixture was 50 ng of template genomic DNA, 1 unit of Taq DNA polymerase, 100 nM of each of the forward and reverse primers, 200 µM of each of the deoxynucleotide triphosphates, and standard PCR buffer, in a final volume of 25 µL. The thermocycling programme was the initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension step at 72°C for 7 minutes. Amplified PCR products were checked by 2% agarose gel electrophoresis and then sent to Macrogen Corporation (Seoul, Republic of Korea) for bidirectional Sanger sequencing. The presence or absence of the G-to-A transition at nucleotide position 1691 of the F5 gene was determined by direct comparison with the G-to-A transition in the reference sequence (GenBank accession AY364535), using the Chromas Pro sequence alignment software (Technelysium Pty Ltd., Queensland, Australia). Phenotypically normal heterozygous and homozygous positive control samples plus wild-type negative controls were sequenced in each batch to validate interpretation. Patients were determined as FVL-positive if the heterozygous or homozygous c.1691G>A variant was identified, and as FVL-negative if it was not.

### *Mutational Subgroup Classification*

Patients were classified into four mutational subgroups based on combined results of MPN panel testing and FVL analysis: (i) FVL-positive/MPN-positive: patients with FVL and at least one of the three MPN driver mutations (n=7); (ii) FVL-positive/MPN-negative (TN-ET with FVL): patients with FVL but no three MPN driver mutations (n=14); (iii) FVL-negative/MPN-positive: patients without FVL but with at least one of the three MPN driver mutations (n=1); and (iv) FVL-negative/MPN-negative: patients without FVL and without any of the three MPN driver mutations (double-negative ET, n=28). This four-group classification allowed for the study cohort to be analyzed for the individual and combined effects of inherited thrombophilia and somatic driver mutations.

### *Statistical Analysis*

Continuous variables were summarized as mean ± standard deviation or median (interquartile range) according to the distribution, assessed using the Shapiro-Wilk test. The Mann-Whitney U test was used for group comparisons, with Benjamini-Hochberg's false discovery rate correction for multiple comparisons. Categorical variables were compared using Fisher's exact test. Associations between Factor V Leiden (FVL)

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status and myeloproliferative disorders (MPN) status were assessed using univariate logistic regression adjusted for age and sex. Because the number of cases in some strata was small, an additional logistic regression using a Firth (dichotomous-reduced) model was performed to obtain stable estimates in the event of possible segregation (16). Spearman's rank correlation coefficient assessed the relationships between continuous variables. A post-hoc statistical power analysis was performed for the main association between FVL and MPN. A p-value < 0.05 was considered statistically significant. Analyses were performed using R (version 4.X) (17).

## Results:

The review included demographic and laboratory variables. The laboratory variables included age, gender, WBC, neutrophils, lymphocytes, monocytes, hemoglobin, platelets, Factor V, and MPN. There were no missing data in regard to the variables, so all 50 patients were considered for the final statistical analysis.

Because of the small sample size, some continuous variables were not normally distributed. Therefore, the descriptive data were analyzed to include either the mean with standard deviation or the median with interquartile range. Comparisons between groups were evaluated with the Mann-Whitney U test for continuous variables and the Fisher's exact test for categorical variables. Adjusted logistic regression was used to evaluate the relationship of Factor V and MPN, with age and gender as covariates.

### General Description of the Study Sample

The investigation analyzed a sample of 50 patients. The demographics and prolonged lab characteristics are outlined in Table 1. The average patient was 59.5 years old, and the average platelet count was 634.79. The interquartile ranges for lab measurements showed variability. The Shapiro-Wilk test indicated that the age variable, lymphocyte %, platelet count, and WBCs were not normally distributed, indicating that the other comparisons should be based on non-parametric statistical tests.

**Table 1:** Continuous demographic and laboratory characteristics of the study sample.

| Variable | N | Mean +/- SD | Median (Q1-Q3) | Minimum | Maximum | Shapiro-Wilk p |
|---|---|---|---|---|---|---|
| **Age (years)** | 50 | 58.54 +/- 7.57 | 59.50 (52.50-65.00) | 45.00 | 70.00 | 0.019 |
| **WBC** | 50 | 10.11 +/- 3.36 | 10.74 (7.58-13.10) | 4.20 | 15.35 | 0.008 |
| **Neutrophils (%)** | 50 | 61.09 +/- 9.05 | 63.27 (53.37-67.10) | 45.93 | 80.66 | 0.172 |
| **Lymphocytes (%)** | 50 | 25.80 +/- 6.40 | 25.94 (22.70-29.00) | 13.40 | 42.00 | 0.024 |
| **Monocytes (%)** | 50 | 6.65 +/- 1.72 | 6.61 (5.55-7.73) | 3.00 | 11.13 | 0.160 |
| **Hemoglobin (Hb)** | 50 | 13.32 +/- 1.69 | 13.33 (12.26-14.25) | 9.50 | 17.50 | 0.528 |
| **Platelets (Plt)** | 50 | 664.41 +/- 177.61 | 634.79 (566.73-767.30) | 385.00 | 1121.00 | 0.028 |

*Note. Q1 = first quartile; Q3 = third quartile; WBC = white blood cell count; Hb = hemoglobin; Plt = platelets. The Shapiro-Wilk p-value assesses normality.*

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Table 2 shows the demographic distribution of the sample. 68.0% of the sample were male, while the remaining 32.0% were female. Factor V testing was positive in 21 patients (42.0% of the sample) and negative in 29 patients (58.0% of the sample). Eight patients (16.0% of the sample) were diagnosed with myeloproliferative disorders, while the remaining 84.0% tested negative.

**Table 2.** Categorical distribution of sex, Factor V status, and MPN status**.**

| Variable | Category | Frequency | Percentage |
|---|---|---|---|
| **Sex** | Male | 34 | 68.0% |
| **Sex** | Female | 16 | 32.0% |
| **Factor V** | Negative | 29 | 58.0% |
| **Factor V** | Positive | 21 | 42.0% |
| **MPN** | Negative | 42 | 84.0% |
| **MPN** | Positive | 8 | 16.0% |

*Note. Percentages were calculated from the total sample size of 50 patients*

**Association Between Factor V and MPN**

A clear statistical association exists between factor V positivity and the presence of myeloproliferative disorders. As shown in Table 3-3, myeloproliferative disorders were present in 7 of 21 factor V-positive patients, compared to only 1 of 29 factor V-negative patients. Fisher's exact test demonstrated that this association was statistically significant ($p = 0.007$). The odds ratio was 14.00, indicating that factor V-positive patients have a significantly higher probability of developing myeloproliferative disorders compared to factor V-negative patients.

Figure 1 illustrates the distribution of myeloproliferative disorders according to factor V status. The figure shows a higher concentration of myeloproliferative disorders in the factor V-positive group, supporting the association shown in Table 3.

**Table 3:** Cross-tabulation of Factor V status and MPN status**.**

| Factor V status | MPN positive | MPN negative | Total |
|---|---|---|---|
| **Positive** | 7 | 14 | 21 |
| **Negative** | 1 | 28 | 29 |
| **Total** | 8 | 42 | 50 |

*Note. Fisher exact test: p = 0.007; odds ratio (OR) = 14.00 (1.52-63.36).*

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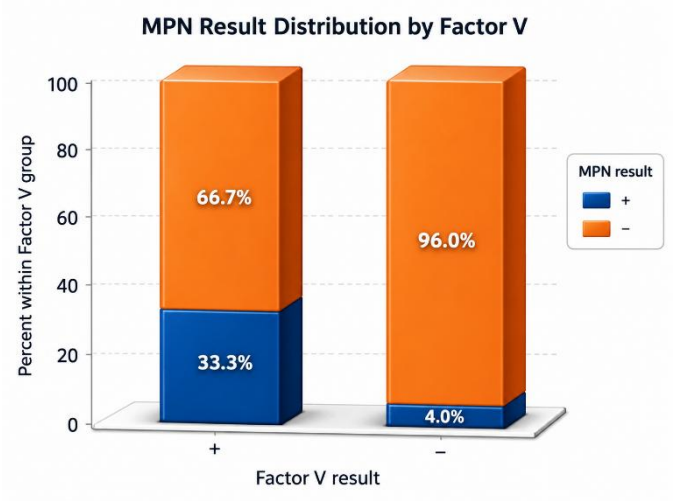


**Figure 1.** Distribution of MPN status according to Factor V status

### Comparison of Patients According to Factor V Status

Table 3 shows the comparison of continuous variables between Factor V-positive and Factor V-negative patients. The application of the false discovery rate (FDR) to control for multiple comparisons showed no statistically significant differences. Despite Factor V-positive patients showing a higher median lymphocyte percentage, the difference was not statistically significant after the FDR adjustment. Age, WBC, and the percentages of neutrophils, monocytes, hemoglobin, and platelet count also did not show statistically significant differences.

Figure 2 shows the distribution of platelet counts according to Factor V status. Although individual variability was present, platelet counts did not differ significantly between the two Factor V groups, consistent with the results presented in Table 4.

**Table 4.** Comparison of continuous variables according to Factor V status.

| Variable | Factor V positive n=21: Median (Q1-Q3) | Factor V negative n=29: Median (Q1-Q3) | Mann-Whitney p | FDR p | Cliff's delta |
|---|---|---|---|---|---|
| **Age (years)** | 60.00 (55.00-65.00) | 56.00 (52.00-65.00) | 0.574 | 0.602 | -0.10 |
| **WBC** | 11.58 (7.58-13.32) | 10.66 (7.58-12.54) | 0.392 | 0.602 | -0.14 |
| **Neutrophils (%)** | 64.27 (54.17-67.40) | 59.62 (53.10-65.17) | 0.602 | 0.602 | -0.09 |
| **Lymphocytes (%)** | 27.38 (24.09-30.62) | 25.08 (22.15-27.38) | 0.075 | 0.526 | -0.30 |
| **Monocytes (%)** | 6.63 (5.83-7.77) | 6.59 (5.20-7.60) | 0.455 | 0.602 | -0.13 |
| **Hemoglobin (Hb)** | 13.41 (12.65-14.53) | 13.05 (12.18-14.05) | 0.409 | 0.602 | -0.14 |
| **Platelets (Plt)** | 612.16 (556.60-750.70) | 641.10 (587.90-781.40) | 0.321 | 0.602 | 0.17 |

*Note. The Mann-Whitney U test was used for group comparisons, and p-values were adjusted using FDR. Cliff's delta represents ffect size according to the coding used in the analysis.*

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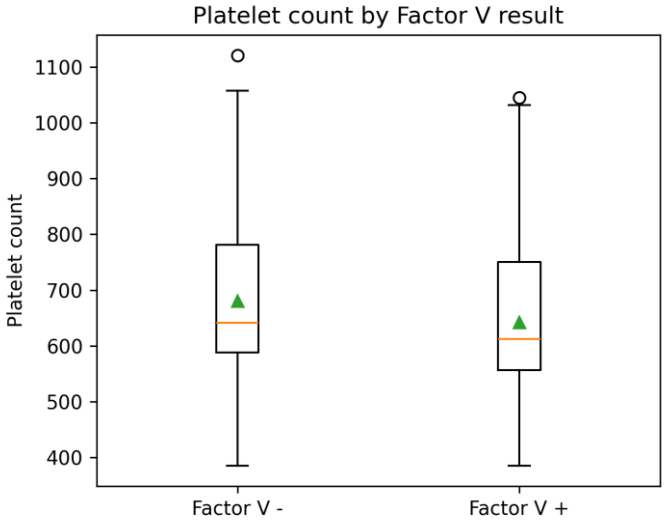


**Figure 2.** Distribution of platelet count according to Factor V status

### Comparison of Patients According to MPN Status

The data in Table 3-5 compares the continuous variables between the MPN-positive and MPN-negative patients. None of the continuous variables had a statistically significant difference after FDR correction. The levels of hemoglobin and the percentage of lymphocytes were both higher among the MPN-positive patients, although these differences were not statistically significant given the current sample size.

**Table 5.** Comparison of continuous variables according to MPN status**.**

| Variable | MPN positive n=8: Median (Q1-Q3) | MPN negative n=42: Median (Q1-Q3) | Mann-Whitney p | FDR p | Cliff's delta |
|---|---|---|---|---|---|
| **Age (years)** | 61.50 (55.00-64.50) | 58.00 (51.25-65.00) | 0.482 | 0.844 | -0.16 |
| **WBC** | 10.29 (5.87-13.50) | 10.74 (7.60-13.04) | 0.979 | 0.979 | -0.01 |
| **Neutrophils (%)** | 64.58 (53.09-66.91) | 62.69 (53.37-67.10) | 0.958 | 0.979 | 0.01 |
| **Lymphocytes (%)** | 27.70 (25.15-30.71) | 25.72 (22.22-28.52) | 0.128 | 0.675 | -0.35 |
| **Monocytes (%)** | 6.50 (5.91-7.37) | 6.61 (5.55-7.79) | 0.895 | 0.979 | -0.03 |
| **Hemoglobin (Hb)** | 13.86 (13.26-14.86) | 13.16 (12.23-14.10) | 0.209 | 0.675 | -0.29 |
| **Platelets (Plt)** | 605.70 (514.05-646.62) | 638.39 (574.10-777.88) | 0.289 | 0.675 | 0.24 |

*Note. The Mann-Whitney U test was used to compare MPN-positive and MPN-negative patients, with FDR adjustment for multiple comparisons.*

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Figures 3 to 6 display age, WBC, hemoglobin, and platelet count by MPN status. These figures substantiate the statistics in Table 4-5. Regarding the continuous variables, we could not find a distinct separation between the MPN-positive and MPN-negative groups.

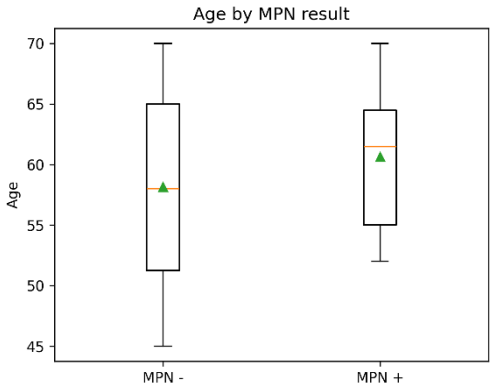


**Figure 3.** Distribution of age according to MPN status.

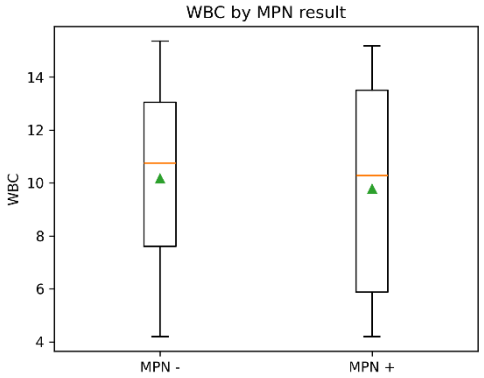


**Figure 4.** Distribution of WBC according to MPN status.

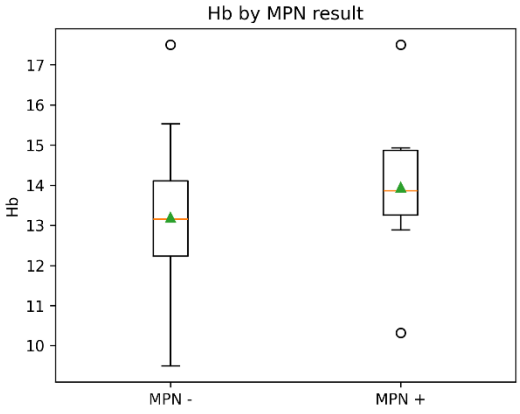


**Figure 5**. Distribution of hemoglobin according to MPN status.

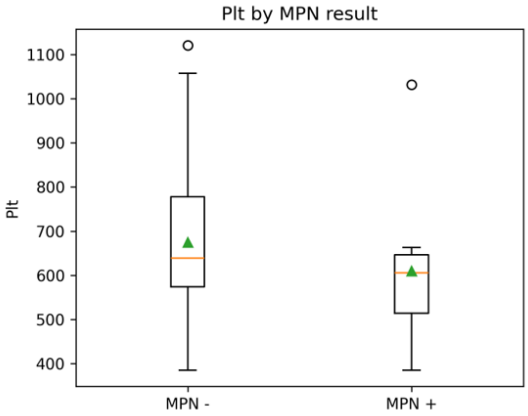


**Figure 6.** Distribution of platelet count according to MPN status.

## Correlations Among Continuous Variables

The relationships between the continuous variables were examined using Spearman's rank correlation coefficient. This analysis revealed a mean negative correlation between age and hemoglobin, indicating that hemoglobin values decreased with advancing age. Furthermore, a positive correlation was found between white blood cell count and neutrophil percentage, and an inverse correlation between white blood cell count and platelet count. Table 6 shows the significant correlations, complemented by Figure 7, which is a heatmap of all Spearman's rank correlation coefficients.

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**Table 6.** Statistically significant correlations among continuous variables.

| First variable | Second variable | Spearman rho | p value | Direction |
|---|---|---|---|---|
| **Age (years)** | Hemoglobin (Hb) | -0.46 | <0.001 | Negative |
| **WBC** | Neutrophils (%) | 0.39 | 0.005 | Positive |
| **WBC** | Platelets (Plt) | -0.37 | 0.009 | Negative |

*Note. Only correlations with $p < 0.05$ are presented. No multiple-comparison correction was applied to this descriptive correlation matrix.*

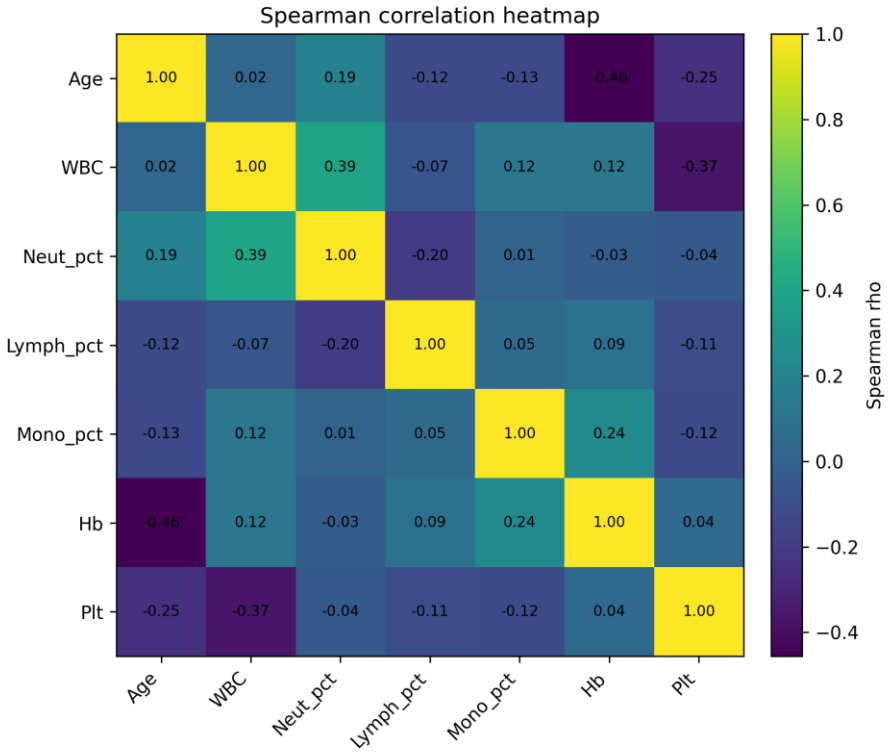


**Figure 7.** Heatmap of Spearman correlation coefficients among continuous variables.

## Logistic Regression Model for Prediction of MPN

Logistic regression models were constructed using multiple myeloproliferative neoplasm (MPN) status as the dependent variable to assess whether the association between factor V (F) and MPN was independent of sex and age. The results of the univariate and adjusted models are presented in Table 7. Univariate analysis showed that factor V positivity was associated with an increased likelihood of developing MPN (odds ratio = 14.00) and was statistically significant. In the model adjusted for sex and age, factor V positivity remained associated with MPN (odds ratio = 13.56, p = 0.022). On the other hand, in the adjusted model, age and sex did not show a statistically significant association with MPN.

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**Table 6.** Logistic regression analysis of factors associated with MPN status.

| Model | Predictor | N | OR | 95% CI | p value |
|---|---|---|---|---|---|
| **Univariable** | Factor V positive | 50 | 14.00 | 1.56-125.26 | 0.018 |
| **Univariable** | Age | 50 | 1.05 | 0.94-1.16 | 0.395 |
| **Univariable** | Male sex | 50 | 0.40 | 0.09-1.86 | 0.243 |
| **Adjusted** | Factor V positive | 50 | 13.56 | 1.46-126.37 | 0.022 |
| **Adjusted** | Age | 50 | 1.06 | 0.93-1.21 | 0.367 |
| **Firth's penalised** | Factor V positive | 50 | 12.89 | 1.46-125.37 | 0.025 |
| **Adjusted** | Male sex | 50 | 0.37 | 0.06-2.18 | 0.269 |

*Note. OR = odds ratio; CI = confidence interval. The adjusted model included Factor V status, age, and sex in the same model.*

In total, this study examines 50 patients. Of these patients, Factor V was positive in 42.0%, and MPN was positive in 16.0%. A statistically significant association between Factor V and MPN was the major finding, with Factor V-positive patients demonstrating a higher prevalence of MPN (odds ratio = 14.00, Fisher exact test). This association also proved to be significant when age and sex were considered, as demonstrated by the logistic regression.

On the contrary, age, WBC, hemoglobin, and platelet count, all continuous variables, even when factoring age and sex, did not demonstrate a statistically significant association with either Factor V or MPN after controlling for multiple comparisons. Specific relationships of laboratory variables were demonstrated in the correlation analysis, the most significant being the association of age and hemoglobin (negative), WBC and % neutrophil (positive) and WBC and platelet count (negative). Among the patients sampled, the Factor V and MPN association presented the most significant statistical finding.

### Post-hoc Power Analysis

A post-hoc power analysis was conducted to assess the statistical power achieved by the study. Given the observed effect size (OR = 14.00 for the association between FVL and MPN status), the actual sample size of 50 patients, an allocation ratio of approximately 1:2.1 (FVL-positive to FVL-negative), and a two-tailed test at $\alpha = 0.05$, the study achieved approximately 82% power to detect the observed association. This exceeds the conventional threshold of 80% and supports the validity of the statistically significant findings reported.

## Discussion

This study examined FVL prevalence and its relationship to MPN driver mutations in fifty Iraqi ET patients, yielding three principal findings. First, FVL was detected in 42.0% of patients — substantially exceeding Eastern population estimates of approximately 7.4% (18). noting that no population-based FVL prevalence study has been conducted specifically in Iraq. FVL allele frequency also shows geographic variation linked to ancient population history, with evidence of elevated frequencies in Near Eastern and European populations (19), which may partly explain the prevalence observed here.

The international Caucasian range of 1–15% (11,20). Second, and most importantly, fourteen FVL-positive triple-negative patients were identified (33.3% of the TN-ET subgroup), directly supporting our study hypothesis that inherited

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thrombophilic factors may represent an under-investigated contributor to the thrombotic phenotype in patients lacking canonical MPN driver mutations. Third, FVL positivity was independently associated with canonical MPN driver mutation presence, with FVL-positive patients demonstrating fourteen-fold higher odds of MPN positivity (OR = 14.00; p = 0.007), an association that remained robust after adjustment for age and sex (OR = 13.56; p = 0.022) and was confirmed by Firth's penalised logistic regression (OR = 12.89; p = 0.025).

These comparisons were made against previously published population estimates rather than a contemporaneous, matched healthy Iraqi control group; the observed elevation should therefore be interpreted as hypothesis-generating.

The elevated FVL prevalence almost certainly reflects ascertainment bias inherent to a tertiary haematology referral cohort, which enriches for patients with overt or impending thrombotic events and thereby over-represents inherited thrombophilic genotypes relative to community prevalence estimates. The high rates of consanguineous marriage in Iraqi society, where studies indicate rates ranging from about 24% to over 60% of marriages in various regional surveys (14, 21), may contribute to an increased likelihood of dominant genetic conditions reaching clinical expression. Nevertheless, the magnitude of the elevation (42.0% versus ~7.4% in the general population) suggests that FVL testing in ET patients is clinically warranted even if the observed prevalence is partially inflated by referral patterns.

The strong positive association between FVL and MPN driver mutation positivity was not the primary hypothesis under investigation; rather, our hypothesis focused on the potential role of FVL as an explanatory factor in the triple-negative subgroup. However, this finding is biologically coherent: JAK2 V617F and related driver mutations promote thrombosis through platelet-mediated and JAK-STAT-driven mechanisms (12,22), while FVL amplifies coagulation cascade activity through APC resistance at the Arg506 cleavage site (10,23). When these mechanistically distinct prothrombotic pathways coexist, their combined burden is more than additive. Patients harbouring both FVL and a canonical driver mutation likely represent the most symptomatic and thrombophilically burdened end of the ET clinical spectrum — and consequently those most likely to be referred for comprehensive molecular evaluation at a tertiary centre, generating the observed positive association. This interpretation is consistent with findings from a comparable Saudi Arabian cohort, where co-mutation of *JAK2 V617F* and FVL was significantly concentrated among patients with thrombotic complications (24).

Among the forty-two triple-negative patients, fourteen (33.3%) were FVL-positive, identifying a clinically important subgroup in whom inherited APC resistance — rather than canonical *JAK-STAT* pathway activation — may represent the dominant prothrombotic mechanism. This finding directly validates our study hypothesis and has immediate clinical implications: for these patients, anticoagulant strategies targeting the coagulation cascade may complement or supersede conventional cytoreductive ET management, though prospective outcome data are required to validate this therapeutic approach (10,25). The comparable haematological profiles between FVL-positive and FVL-negative triple-negative patients (Table 3-4) further support the notion that FVL operates through coagulation dysregulation rather than

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myeloproliferative mechanisms in TN-ET patients. No statistically significant haematological differences were observed between FVL-positive and FVL-negative patients, or between MPN-positive and MPN-negative patients after multiple comparison correction. This is expected: FVL operates exclusively within the coagulation cascade and exerts no direct effect on platelet production, erythropoiesis, or leucopoiesis (10,23,26). The significant correlations revealed by the Spearman test—an inverse relationship between age and hemoglobin level (rho = -0.46), a positive relationship between white blood cells and neutrophils (rho = 0.39), and an inverse relationship between white blood cells and platelets (rho = -0.37)—are well-established hematological associations in cases of essential thrombocythemia (ET) and align with data published in medical records (7,12).

The findings collectively support. These preliminary findings suggest that FVL testing may warrant consideration in Iraqi ET practice regardless of driver mutation status, pending confirmation in larger, prospective, multicentre studies.

To identify MPN-positive patients at heightened thrombotic risk warranting intensified management, and to identify FVL-positive TN-ET patients in whom inherited thrombophilia may be the principal pathogenetic determinant — a subgroup that would otherwise remain undetected under standard molecular workup limited to canonical driver mutations. This proposal should be regarded as hypothesis-generating and requires prospective validation before informing routine testing recommendations.

**Limitations of the study:**

The principal limitations are the retrospective single-center design, the modest sample size, the absence of longitudinal clinical outcome data, and the lack of a healthy control group from the same population, all of which constrain causal inference and generalizability. A further important limitation is the absence of a contemporaneous healthy Iraqi control group tested using the same PCR-Sanger methodology. Without such a control group, the claimed elevation in FVL prevalence relative to the general population cannot be formally validated, and comparisons with population prevalence estimates remain methodologically indirect. Additionally, the retrospective design precluded systematic capture of documented prior thrombotic event history — the primary clinical indication for FVL testing in practice — which could not be systematically retrieved from the available records.

Additionally, the high proportion of triple-negative patients (84.0%) exceeds the 10–20% reported in Western cohorts (6,7), which may reflect technical limitations in the detection sensitivity of the TRUPCR® MPN Panel Kit (analytical sensitivity 1% mutant allele frequency), the absence of confirmatory Sanger sequencing for MPN mutations, or genuine population-level differences in the molecular landscape of ET in Iraq. Future studies should employ next-generation sequencing panels with higher sensitivity to reassess the true prevalence of low-burden driver mutations in this population. The wide confidence intervals observed in the logistic regression models (e.g., 1.46–126.37) reflect the small number of MPN-positive cases and underscore the need for larger confirmatory studies. Nevertheless, the application of Firth's penalised regression and the post-hoc power analysis (82% power) provide reassurance that the observed association is unlikely to be a statistical artefact.

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## Conclusion

This study presents the first systematic characterization of the factor V Leiden (FVL) mutation in Iraqi patients with essential thrombocythemia, demonstrating a significantly higher prevalence of FVL compared to previously published estimates (pending confirmation by a healthy control group) and a strong, independent association between FVL and the positivity of the mutation causing myeloproliferative disorders. The identification of fourteen FVL-positive and two triple-negative patients (33.3% of the triple-negative subgroup of essential thrombocythemia) directly supports our hypothesis that inherited coagulation dysregulation may represent a clinically significant and currently understudied pathogen in this patient population. The mechanistic synergy between JAK-STAT-dependent coagulation pathways and APC resistance suggests that FVL-positive myeloproliferative disorders (FPDs) represent a high-risk subgroup warranting intensified anticoagulation, while FVL-positive patients with triple-negative results highlight the need to broaden molecular testing beyond traditional causative mutations in the management of primary thrombocythemia in Iraq. Future studies incorporating clinical outcome data, population-level control groups, and more sensitive mutation detection methods are essential to fully determine the prognostic significance of these findings.

**Ethical Considerations:** This study received ethical approval from the Department of Scientific Affairs, College of Medicine, Iraqi University, Baghdad, Iraq (Reference No. FM.SA16, February 3, 2026). The studies were conducted in accordance with the Declaration of Helsinki (February 3, 2026). All participants (or their legal guardians, if applicable) provided written informed consent before their data were used in this study.

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