Research Square

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Erythropoietin levels in differentiating primary and secondary erythrocytosis, and correlation with JAK2 positivity: a retrospective chart review

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Abstract

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm characterized by clonal erythrocytosis. Differentiating PV from secondary erythrocytosis is essential because the management and prognosis differ significantly. Although subnormal erythropoietin (EPO) is a minor criterion in the World Health Organization (WHO) diagnosis of primary PV, its reliability as a discriminator from secondary erythrocytosis remains suboptimal. Despite limited reliability, EPO continues to be used as a screening test, primarily due to lower cost and quicker turnaround relative to Janus kinase 2 (JAK2) testing. To evaluate how well EPO levels correlate with JAK2 status, we conducted a single-center retrospective review of 388 patients who underwent JAK2 mutation testing between 2017 and 2019. Of these, 9.5% (n = 37) were JAK2-positive. Among JAK2-positive patients with available EPO levels (n = 18), 44.4% had values within the normal range. Receiver operating characteristic analysis showed fair discriminative ability of EPO for JAK2 mutation status (area under the curve 0.7355). An optimal cutoff of ≤ 6.0 mU/mL yielded a sensitivity of 68.8% and specificity of 83.3%, but with substantial overlap between groups. Nearly half of JAK2-positive patients had normal EPO levels, underscoring that EPO is an unreliable standalone diagnostic marker for primary PV or JAK2-positive disease. JAK2 mutational analysis remains the most accurate and clinically meaningful test. Routine measurement of EPO may add cost without improving diagnostic accuracy, particularly in populations with confounders such as those living at high altitudes or those with comorbid hypoxic conditions.

Introduction

Polycythemia vera (PV) is a chronic myeloproliferative neoplasm characterized by clonal proliferation of myeloid lineages [1]. The hallmark hematologic feature of the disease is erythrocytosis, defined as an increase in hemoglobin (Hb) concentration, hematocrit (Hct), or both. PV is associated with an increased risk of arterial and venous thromboembolism, along with a cumulative risk of progression to myelofibrosis or acute leukemia. In contrast, current clinical studies are lacking as to whether potential adverse outcomes are seen with secondary erythrocytosis, including but not limited to hypoxia-induced erythrocytosis (cardiac disease, pulmonary disease, smoking) and iatrogenic forms (supplemental testosterone). These issues are primarily managed by addressing the underlying condition driving erythropoiesis. Accurate distinction between primary and secondary erythrocytosis is essential during diagnostic evaluation, as the two differ markedly in management, long-term surveillance, and associated risks.

The identification of the Janus kinase 2 (*JAK2*) V617F mutation in 2005 marked a major advancement in the understanding of PV and led to significant changes in its diagnostic framework, changes that are now incorporated into the current World Health Organization (WHO) classification criteria [2]. More than 95% of patients harbor an acquired activating mutation in the *JAK2* gene, most commonly the V617F substitution in exon 14, which constitutes a major diagnostic criterion [3, 4]. Prior to this discovery, low erythropoietin (EPO) levels were the predominant markers used to help differentiate PV from secondary erythrocytosis, based on the principle that autonomous erythropoiesis in PV suppresses EPO production.

According to the current WHO guidelines, a diagnosis of PV is established when all three major criteria are met, or when the first two major criteria are present in conjunction with the minor criterion (Table 1). The three major criteria are: 1) elevated Hb (>16.5 g/dL in men, >16.0 g/dL in women) or Hct (>49% in men, >48% in women) or increased red cell mass, 2) bone marrow biopsy showing hypercellularity with panmyelosis (prominent erythroid, granulocytic, and megakaryocytic proliferation), and 3) presence of *JAK2* V617F or *JAK2* exon 12 mutation. The minor criterion is a subnormal serum EPO level [5, 6].

Table 1. WHO 2022 Diagnostic Criteria for Polycythemia Vera

Criteria	Diagnostic Features
Major Criteria	1. **Elevated hemoglobin or hematocrit:** • Hemoglobin > 16.5 g/dL (men) or > 16.0 g/dL (women) • Hematocrit > 49% (men) or > 48% (women)
	2. **Bone marrow biopsy** showing age-adjusted hypercellularity with trilineage proliferation (panmyelosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes
	3. Presence of ***JAK2* V617F or *JAK2* exon 12 mutation**
Minor Criteria	1**. Subnormal serum erythropoietin level**

Diagnosis of polycythemia vera requires all 3 major criteria or the first 2 major criteria and the minor criterion. Hemoglobin and hematocrit thresholds are per WHO 2022 criteria.

In clinical practice, serum EPO levels are commonly ordered as part of the initial diagnostic algorithm in undifferentiated erythrocytosis [7]. This is largely due to cost and quicker results compared to JAK2 testing. A concern with this approach is the ongoing uncertainty regarding the reliability of EPO levels as a screening tool to differentiate primary from secondary erythrocytosis accurately, or to definitively rule out PV. The use of EPO as a diagnostic criterion is derived from older studies predating the discovery of the JAK2 mutation [8]. Relying solely on serum EPO levels to distinguish between primary and secondary erythrocytosis is problematic, as normal or borderline EPO levels may be seen in both JAK2-positive and negative patients, leading to diagnostic uncertainty and potential delays in appropriate workup or inappropriately ruling out PV because EPO levels are not subnormal.

For this reason, we conducted a retrospective chart review to evaluate whether serum EPO levels can accurately predict JAK2 status and if there is an EPO threshold that may aid in this distinction between clonal and nonclonal erythrocytosis.

Methods

We conducted a single-center, retrospective study at our institution, reviewing electronic medical records to identify adult patients who underwent JAK2 mutational analysis between August 2017 and July 2019. Patients were identified based on charges generated for JAK2 mutation laboratory testing. For each individual, we collected data on EPO levels, peripheral blood counts (including white blood cell count, Hb, Hct, and platelet count), serum creatinine, liver function tests, ordering provider, and relevant comorbidities. Comorbidities included a history of thrombosis, cardiovascular or pulmonary disease, supplemental testosterone use, diuretics, supplemental oxygen, and smoking status. Patients with a known JAK2-positive myeloproliferative neoplasm before the time of repeat JAK2 mutation testing were excluded. A total of 388 patients met the inclusion criteria for the study.

Statistical Analysis

We summarized baseline demographic, clinical, and laboratory characteristics for the overall cohort and stratified measures by JAK2 mutation status (Table 1). Continuous variables, including age, white blood cell count, Hb, Hct, platelet count, creatinine, liver function tests, and EPO levels, were compared using analysis of variance or Wilcoxon Rank Sum testing. Categorical variables such as sex, history of thrombosis, cardiovascular disease, pulmonary disease, oxygen use, smoking status, testosterone and diuretic use, subspecialty referral, and clot type (arterial, venous, mesenteric) were compared using chi-square or Fisher's exact tests. Derived hematologic variables, including erythrocytosis (defined by Hct and Hb thresholds, stratified by sex), leukocytosis, and thrombocytosis, were also evaluated. Receiver operating characteristic (ROC) analysis was performed to evaluate the ability of ln(EPO) levels to discriminate JAK2 mutation status and diagnostic accuracy (Figure 1). The area under the ROC curve (AUC) was calculated, and statistical significance was assessed using a Chi-square test. Youden's J statistic was applied across a range of ln(EPO) thresholds to identify the optimal cutoff point and corresponding sensitivity and specificity for predicting JAK2 mutation status (Table 2). The distribution of ln(EPO) values was further compared between JAK2-positive and JAK2-negative patients, with summary statistics including mean, minimum, maximum, and standard deviation calculated to characterize group differences (Figure 2). Optimal cutoff points were also determined for continuous hematologic parameters (Hb, Hct, white blood cell count, and platelet count) using the AUC and Youden's J method when the AUC was statistically significant (Table 3). We examined the relationships between serum EPO levels and clinical, hematologic, and thrombotic outcomes using univariate logistic regression (Table 4). Both continuous EPO values and categorical EPO levels (≤6 vs. >6 mU/mL) were evaluated.

Results

A total of 388 patient charts were reviewed who underwent JAK2 mutational analysis between August 2017 and July 2019 (Table 1). Age, sex, serum EPO levels, peripheral blood counts, serum creatinine, liver function tests, and patient comorbidities were recorded and compared between JAK2-positive and JAK2-negative patients. Of these patients, 9.5% (n = 37) were JAK2-positive and 90.5% (n = 351) were JAK2-negative. Of the JAK2-positive patients, 48.6% (n = 18) had serum EPO levels measured. Among

those, 44.4% (n = 8) had EPO levels within the normal reference range (4–27 mU/mL), indicating that nearly half of JAK2-positive individuals did not exhibit the expected subnormal EPO suppression. Among JAK2-negative patients, 142 had serum EPO levels measured; of these, 81.7% (n = 116) had EPO values within the normal reference range, 7.7% (n = 11) had subnormal values (< 4 mU/mL), and 10.6% (n = 15) had elevated levels (> 27 mU/mL). Age was significantly associated with JAK2 mutation status, with JAK2-positive individuals being older on average than JAK2-negative individuals (mean age 61.9 vs. 50.9 years; $p < 0.0001$). Sex distribution was similar between groups (JAK2-positive: 40.5% male, 59.5% female; JAK2-negative: 54.4% male, 45.3% female). JAK2-positive patients demonstrated significantly higher white blood cell counts (mean 14.08 vs. 9.31 $\times 10^3/\mu L$; $p < 0.0001$), platelet counts (mean 533.32 vs. 290.74 $\times 10^3/\mu L$; $p < 0.0001$), with increased prevalence of leukocytosis (43.2% vs. 19.7%; $p = 0.001$) and thrombocytosis (64.9% vs. 18.2%; $p < 0.0001$). Mean serum EPO levels were comparable in absolute terms (42.29 vs. 35.44 mU/mL) but demonstrated substantial variability, with significantly different distributions between groups ($p = 0.0009$). Cardiovascular disease was more common in the JAK2-positive group (45.9% vs. 26.1%; $p = 0.0105$), while other comorbidities, thrombotic history, and medication use were similar between groups. Mean Hb and Hct levels were marginally lower in JAK2-positive patients compared to JAK2-negative patients (Hb: 14.57 vs. 15.63 g/dL; $p = 0.0521$; Hct: 44.35 vs. 46.71; $p = 0.1414$), with considerable variability within both groups. There were no significant associations between JAK2 mutation status and sex, Hct, serum creatinine, liver function tests, or other comorbidities.

To evaluate the ability of serum EPO levels to distinguish JAK2 mutation status, an ROC curve analysis was performed (Fig. 1). The ROC analysis yielded an AUC of 0.7355, indicating fair discriminative ability of EPO level in predicting JAK2 mutation status. Youden's J statistic was calculated across the ROC curve to determine the optimal EPO threshold for distinguishing JAK2 mutation status, identifying an EPO level of 6.0 mU/mL as the best cutoff (Table 2). At this threshold, sensitivity was 68.8%, specificity was 83.3%, and Youden's J score was 0.521. Lower cutoffs increased specificity but reduced sensitivity, whereas higher cutoffs improved sensitivity at the cost of specificity. Figure 2 shows the distribution of log-transformed EPO values stratified by JAK2 mutation status. The mean ln(EPO) was lower in JAK2-positive patients (1.79 ± 1.55, range 0–6.43) compared to JAK2-negative patients (2.49 ± 1.00, range 0–7.44), consistent with suppressed EPO levels being seen more commonly in JAK2-positive individuals. However, a wide range and overlap of values were observed in both groups. White blood cell count, hemoglobin, and platelet count demonstrated statistically significant but fair ability to predict JAK2 mutation status, with AUCs of 0.699, 0.616, and 0.740, respectively (Table 3). Youden's J statistic was also applied to continuous laboratory measures to assess their ability to distinguish JAK2-positive from JAK2-negative patients. WBC showed an optimal cutoff of 8.60 × $10^3/\mu L$ with sensitivity 81.1% and specificity 54.7% (Youden's J = 0.358). Hb had a cutoff of 17.1 g/dL with sensitivity 86.5% and specificity 33.8% (Youden's J = 0.203). Platelet count demonstrated a cutoff of 442 × $10^3/\mu L$ with sensitivity 64.9% and specificity 85.4% (Youden's J = 0.503). Hct and creatinine did not demonstrate significant discrimination, and no cutoffs were determined. While several hematologic parameters demonstrate

some ability to distinguish JAK2 mutation status, their diagnostic utility is limited by modest accuracy and susceptibility to multiple confounding factors.

Table 4 shows that when modeled as a continuous variable, EPO was not significantly associated with most clinical outcomes or comorbidities. However, when analyzed categorically (EPO ≤ 6 vs. >6 mU/mL), low EPO was strongly associated with reduced odds of Hct-defined erythrocytosis in both men (OR = 0.31, 95% CI: 0.17–0.56, p < 0.0001) and women (OR = 0.23, 95% CI: 0.11–0.47, p < 0.0001), as well as Hb-defined erythrocytosis in men (OR = 0.42, 95% CI: 0.23–0.77, p = 0.0007) and women (OR = 0.48, 95% CI: 0.23–0.98, p = 0.0007). Low EPO was also associated with increased odds of leukocytosis (OR = 2.25, 95% CI: 1.25–4.08, p = 0.0073), thrombocytosis (OR = 6.99, 95% CI: 3.12–15.67, p < 0.0001), prior thrombosis (OR = 2.22, 95% CI: 1.34–3.68, p = 0.0021), and mesenteric clot (OR = 16.0, 95% CI: 2.16–118.5, p = 0.0067). These findings suggest that categorical low EPO may discriminate clinically relevant hematologic phenotypes better than continuous EPO values.

Discussion

In this study, we evaluated the diagnostic utility of serum EPO levels for predicting JAK2 mutation status in patients undergoing mutational testing as part of their erythrocytosis workup. We found that serum EPO levels are not a reliable standalone marker for predicting JAK2 mutation status. Among JAK2-positive patients with available EPO measurements, nearly half had EPO values within the normal range. Among JAK2-negative patients with EPO measured, most values were also within the normal range (81.7%), with 7.7% subnormal and 10.6% elevated. This substantial overlap between groups demonstrates that normal or even low EPO values can occur regardless of underlying clonal or secondary erythrocytosis. Consequently, EPO levels alone provide limited information for differentiating PV from non-clonal erythrocytosis, as normal EPO is common in both populations and subnormal EPO is not exclusive to patients with PV. An optimal EPO cutoff of ≤ 6.0 mU/mL demonstrated only moderate sensitivity (68.8%) and specificity (83.3%), with substantial overlap between JAK2-negative patients. Using this cutoff, the false-negative rate is 31.2%, resulting in nearly one-third of JAK2-positive patients being underdiagnosed by EPO screening alone. Using a lower EPO cutoff of 3 mU/mL, EPO becomes highly specific (94.9%) for PV, but sensitivity decreases (43.8%), consistent with findings also seen in the literature.

Utilization of EPO levels to differentiate between JAK2-mutated and non-clonal erythropoiesis has been a standard approach in clinical practice. However, as our study shows, there is considerable variability in EPO levels regardless of underlying cause, limiting its diagnostic utility.

EPO production is affected by a variety of factors, including chronic hypoxic conditions [9], smoking [10], and altitude [11], which can confound the interpretation of EPO levels. Our study population resides in the Denver, Colorado, metro region, at a moderate-high altitude that can independently stimulate erythropoiesis. This factor is unique compared to most prior studies conducted at or near sea level and may partially explain the observed variability in serum EPO levels among JAK2-positive patients.

Furthermore, serum EPO demonstrates diurnal variation, with levels fluctuating depending on the time of day at which the sample is obtained [12]. In addition to these confounders, the lack of EPO suppression in some JAK2-positive patients may reflect heterogeneity in PV biology, with possible lower allele burden associated with less EPO suppression [13], [14]. While subnormal EPO may support a diagnosis of PV, it remains unreliable as a standalone discriminator between primary and secondary erythrocytosis, though it may still be a reasonable surrogate in settings where JAK2 testing is unavailable. Conversely, a normal EPO level lacks specificity to rule out the presence of the JAK2 mutation.

Interestingly, when serum EPO was analyzed categorically using a threshold of ≤ 6 mU/mL, low levels were strongly associated with reduced odds of meeting standard hematologic definitions of erythrocytosis. This effect was observed across both sexes. Such findings suggest that suppressed EPO may reflect dysregulated feedback rather than absolute red cell mass, and therefore should not be over-interpreted as a marker of erythrocytosis. These results further highlight the complexity of interpreting EPO in the diagnostic evaluation of PV, as physiologic and clonal drivers of erythropoiesis do not always align with serum EPO levels.

Our findings align with recent studies showing that suppressed EPO levels are neither consistently observed in JAK2-positive patients nor sufficiently discriminatory to reliably differentiate PV from secondary erythrocytosis. In a study by Lupak et al. [10], among 75 patients with PV, 68% (n = 51) had subnormal EPO levels while 32% (n = 24) fell within the normal range. Using an EPO cutoff of 3.7 mU/mL, they reported a sensitivity of 68% and a specificity of 93.7%. Subnormal EPO levels were significantly associated with PV (OR 0.857, $p < 0.001$); however, when JAK2 mutation status was known, EPO levels did not provide additional diagnostic value (OR 0.962, $p = 0.269$). Another study conducted by Varda and Tomassetti [15] at Harbor-UCLA Medical Center performed a retrospective chart review between 2015 and 2021 on 141 patients, with 8.5% (n = 12) having PV and the *JAK2* V617F mutation. Of those, 55% (n = 6) had subnormal EPO levels meeting the WHO minor criteria at the time of diagnosis, indicating that nearly half of PV patients had EPO levels within or above the normal range. Chin-Yee et al. [16] also showed that normal EPO levels can be seen in over a third of PV patients. Similarly, Davila-Gonzalez et al. surveyed 577 patients with erythrocytosis, of whom the majority (n = 351) carried a *JAK2* V617F mutation and diagnosis of PV. In comparative ROC analysis, the *JAK2* V617 mutation demonstrated a significantly higher diagnostic accuracy than serum EPO level (AUC 0.897 vs. 0.677), supporting its superiority as a diagnostic marker for PV and reinforcing the limited utility of EPO alone. In their study, an EPO level below 2 mIU/mL was highly specific (> 99%) but showed poor sensitivity (12%), missing the vast majority of PV patients [17].

JAK2 V617F mutational analysis has demonstrated excellent diagnostic accuracy and remains the most reliable tool for confirming PV [18]. In clinical practice, reliance on subnormal EPO levels may lead to underdiagnosis of PV, particularly in patients with borderline Hb or Hct values or comorbid conditions that elevate EPO levels. However, cost and testing availability may limit its routine use, leading clinicians to rely on serum EPO levels as a more accessible, though less accurate, alternative. On the contrary, checking both adds to cost without adding diagnostic value compared to checking JAK2 alone [15].

Our study has several limitations inherent to its retrospective design. The analysis was limited to data available in the electronic medical record, which may be incomplete or inconsistently recorded. EPO levels were only measured in a subset of JAK2-positive patients, introducing potential selection bias and limiting generalizability. The timing of EPO collection was not standardized, and physiologic diurnal variation, as well as the high-altitude location of our patient population, may have contributed to variability in measured levels. Additionally, we were unable to control for confounding variables inherent to a retrospective study. As a single-center study, the findings may also reflect local practice patterns and patient demographics, limiting broader applicability. Despite these limitations, our study adds to the growing consensus and literature that EPO levels have limited utility in distinguishing primary and secondary erythrocytosis and are poor at predicting JAK2 mutational status.

Future studies should prospectively validate EPO thresholds in large cohorts to distinguish primary from secondary erythrocytosis, with stratification by comorbid conditions and by altitude. In addition, investigating the relationship between *JAK2* V617F allele burden and EPO levels could provide important insights into PV biology, as allele burden may correlate with the degree of EPO suppression and disease phenotype [19] [20]. This would help clarify the utility of EPO as a diagnostic marker, optimize threshold selection, and potentially refine algorithms for differentiating primary from secondary erythrocytosis. With the increasing use of JAK2 testing in the evaluation of erythrocytosis, routine measurement of serum EPO as an initial diagnostic screen may simply add to diagnostic uncertainty and delays in care as well as costs. Given evolving evidence regarding the limited utility of EPO levels and the reliability of JAK2 testing, revisions to the diagnostic criteria for PV may be expected in the near future.

Conclusion

In conclusion, our study demonstrates that serum EPO levels have limited reliability in distinguishing PV from secondary erythrocytosis or predicting JAK2 mutation status. Nearly half of JAK2-positive patients had EPO values within the normal range, and although an EPO threshold of ≤ 6.0 mU/mL showed moderate sensitivity and specificity, substantial overlap with JAK2-negative patients limits its diagnostic utility as a standalone marker. *JAK2 V617F* mutational analysis remains the most accurate and clinically meaningful test for confirming PV. Routine measurement of EPO in the era of widespread JAK2 testing may add cost without improving diagnostic accuracy. Future studies should focus on prospective validation of EPO thresholds, the impact of comorbidities and altitude, and the relationship between JAK2 allele burden and EPO levels, which may refine understanding of disease biology and inform potential updates to PV diagnostic criteria.

Abbreviations

AUC
area under the curve
JAK2
Janus kinase 2

Hct
hematocrit
Hb
hemoglobin
PV
polycythemia vera
ROC
Receiver Operating Characteristic
WHO
World Health Organization

Declarations

Competing Interests

The authors declare no competing interests.

Ethics Approval

This retrospective chart review study involving human participants was reviewed and approved by the Institutional Review Board at the University of Colorado Anschutz Medical Campus. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent to Participate

The requirement for informed consent was waived by the Institutional Review Board due to the retrospective nature of the study.

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Author Contribution

W.S., J.R., D.A., and B.M. were responsible for data acquisition and project design. D.A. performed the statistical analyses. M.L.R. interpreted the data and drafted the main manuscript. B.M. supervised the project and provided critical revisions. All authors contributed to the manuscript, reviewed its content, and approved the final version.

Data Availability

The data that support the findings of this study are not publicly available due to patient privacy and institutional restrictions but are available from the corresponding author upon reasonable request and with appropriate institutional approval.

References

1. Spivak JL (2002) Polycythemia vera: myths, mechanisms, and management. Blood 100(13):4272–4290. https://doi.org/10.1182/blood-2001-12-0349
2. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR et al (2005) A Gain-of-Function Mutation of JAK2 in Myeloproliferative Disorders. N Engl J Med 352(17):1779–1790. https://doi.org/10.1056/NEJMoa051113
3. Wang YL, Vandris K, Jones A, Cross NCP, Christos PJ, Adler KP et al (2008) JAK2 Mutations are present in all cases of polycythemia vera. Leukemia 22(6):1289. https://doi.org/10.1038/sj.leu.2405047
4. Tremblay D, Kremyanskaya M, Mascarenhas J, Hoffman R (2025) Diagnosis and Treatment of Polycythemia Vera: A Review. JAMA 333(2):153. https://doi.org/10.1001/jama.2024.20377
5. Barbui T, Thiele J, Gisslinger H, Finazzi G, Vannucchi AM, Tefferi A (2018) The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. Blood Cancer J 8(2):15. https://doi.org/10.1038/s41408-018-0054-y
6. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF et al (2022) The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia 36(7):1703–1719. https://doi.org/10.1038/s41375-022-01613-1
7. Tefferi A (1999) Diagnosing Polycythemia Vera: A Paradigm Shift. Mayo Clin Proc. ;74(2):159–162. https://doi.org/10.4065/74.2.159
8. Messinezy M, Westwood NB, Woodcock SP, Strong RM, Pearson TC (1995) Low serum erythropoietin–a strong diagnostic criterion of primary polycythaemia even at normal haemoglobin levels. Clin Lab Haematol 17(3):217–220
9. Haase VH (2010) Hypoxic regulation of erythropoiesis and iron metabolism. Am J Physiol Ren Physiol 299(1):F1–F13. https://doi.org/10.1152/ajprenal.00174.2010
10. Lupak O, Han X, Xie P, Mahmood S, Mohammed H, Donthireddy V (2020) The role of a low erythropoietin level for the polycythemia vera diagnosis. Blood Cells Mol Dis 80:102355. https://doi.org/10.1016/j.bcmd.2019.102355
11. Faura J, Ramos J, Reynafarje C, English E, Finne P, Finch CA (1969) Effect of Altitude on Erythropoiesis. Blood 33(5):668–676. https://doi.org/10.1182/blood.V33.5.668.668
12. Cahan C, Decker MJ, Arnold JL, Washington LH, Veldhuis JD, Goldwasser E et al (1992) Diurnal variations in serum erythropoietin levels in healthy subjects and sleep apnea patients. J Appl Physiol

72(6):2112–2117. https://doi.org/10.1152/jappl.1992.72.6.2112

13. Dupont S, Masse A, James C, Teyssandier I, Le Couédic JP, Larbret F et al (2007) The JAK2 617V > F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. Blood 110(3):1013–1021. https://doi.org/10.1182/blood-2006-10-054940
14. Chen CC, Chen JL, Lin AJH, Yu LHL, Hou HA (2024) Association of JAK2V617F allele burden and clinical correlates in polycythemia vera: a systematic review and meta-analysis. Ann Hematol 103(6):1947–1965. https://doi.org/10.1007/s00277-024-05754-4
15. Varda MM, Tomassetti S (2022) Erythropoietin Levels in Patients with Polycythemia Vera and Secondary Erythrocytosis. Blood 140(Suppl 1):12181–12182. https://doi.org/10.1182/blood-2022-167756
16. Chin-Yee B et al (2022) Serum erythropoietin levels in 696 patients investigated for erythrocytosis with JAK2 mutation analysis. Am J Hematol 97(4). https://doi.org/10.1002/ajh.26471
17. Davila-Gonzalez D et al (2021) Diagnostic Performance of Erythropoietin Levels in Polycythemia Vera: Experience at a Comprehensive Cancer Center. Clin Lymphoma Myeloma Leuk 21(4):224–229. https://doi.org/10.1016/j.clml.2020.11.002
18. Ancochea A et al (2014) The role of serum erythropoietin level and JAK2 V617F allele burden in the diagnosis of polycythaemia vera. Br J Haematol 167(3):411–417. https://doi.org/10.1111/bjh.13047
19. Goldman JJ, Vandris K, Adriano F, Bourla M, Silver RT (2009) Normal Serum-Erythropoietin (S-epo) Level at Diagnosis of Polycythemia Vera (PV) Correlates with Low JAK2V617F Mutant Allele Burden and Indicates Mild Phenotype. Blood 114(22):4978. https://doi.org/10.1182/blood.V114.22.4978.4978
20. Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A (2007) Prospective identification of high-risk polycythemia vera patients based on JAK2V617F allele burden. Leukemia 21(9):1952–1959. https://doi.org/10.1038/sj.leu.2404854

Tables

Table 1. Summary Measures of Variables for All Data and by JAK2 Status.

Variable	All Data	JAK2 Positive	JAK2 Negative	p-value
N (Sample Size)	388	37	351	--
% of Total Sample	100%	9.5%	90.5%	--
Age (in years)				
Min	19	32	19	**p < 0.0001**
Max	92	92	90	(AOV analysis)
Mean	52.0	61.9	50.92	
Std. Dev.	15.95	16.39	15.56	
Sex				
M	206 (53.1%)	15 (40.5%)	191 (54.4%)	p = 0.2518
F	181 (46.7%)	22 (59.5%)	159 (45.3%)	(Chisquare)
M→ F	1 (0.3%)	0 (0%)	1 (0.3%)	
WBC				
Min	0.9	5.2	0.9	**p < 0.0001**
Max	108.4	108.4	78.3	(AOV Analysis)
Mean	9.77	14.08	9.31	log-transformed data
Std. Dev.	8.12	16.47	6.53	
HGB				
Min	19.9	6.8	6.2	p = 0.0521
Max	22.0	19.3	22	(AOV Analysis)
Mean	15.50	14.57	15.63	
Std. Dev.	3.16	3.11	3.14	
HCT				
Min	19.9	21.6	19.9	p = 0.1414
Max	66	60.9	83.4	(AOV Analysis)
Mean	46.43	44.35	46.71	
Std. Dev.	9.09	9.85	9.16	
Plts				

Min	29.0	44.0	29.0	**p < 0.0001**
Max	2283.0	1260.0	2283.0	(AOV Analysis)
Mean	313.99	533.32	290.74	log-transformed data
Std. Dev.	225.57	312.57	201.32	
Cr				
Min	0	0.45	0	p = 0.1859
Max	14.76	5.04	14.76	(AOV Analysis)
Mean	1.11	1.21	1.10	log-transformed data
Std. Dev.	1.34	0.90	1.38	
LFTs				
0	220 (56.7%)	18 (48.7%)	202 (57.5%)	p = 0.5657
1	110 (28.3%)	12 (32.4%)	98 (27.9%)	(Chisquare)
NT	58 (15.0%)	7 (18.9%)	51 (14.5%)	
EPO				
Min	0	0	0	p = 0.0009
Max	1706	618	1706	(Wilcoxon Rank Sum)
Mean	36.18	42.29	35.44	
Std. Dev.	159.21	148.5	160.93	
Thrombosis Hx				
0	263 (68.1%)	21 (56.8%)	242 (69.3%)	p = 0.1183
1	123 (31.9%)	16 (43.2%)	107 (30.7%)	(Chisquare)
CVD				
0	278 (72.0%)	20 (54.1%)	258 (73.9%)	**p = 0.0105**
1	108 (28.0%)	17 (45.9%)	91 (26.1%)	(Chisquare)
Pulm Dis				
0	253 (65.5%)	26 (70.3%)	227 (65.0%)	p = 0.5246
1		11 (29.7%)	122 (35.0%)	(Chisquare)

	133 (34.5%)			
T use				
0	364 (93.8%)	37 (100%)	327 (93.2%)	p = 0.1495
1	24 (6.2%)	0 (0%)	24 (6.8%)	(Fisher's Exact Test)
Diuretic use				
0	301 (77.6%)	27 (73.0%)	274 (78.1%)	p = 0.4802
1	87 (22.4%)	10 (27.0%)	77 (21.9%)	(Chisquare)
O2				
0	342 (88.1%)	30 (81.1%)	312 (88.9%)	p = 0.0505
1	45 (11.6%)	6 (16.2%)	39 (11.1%)	(Fisher's Exact Test)
2	1 (0.3%)	1 (2.7%)	0 (0%)	
Subspecialty				
Hematology	294 (75.8%)	32 (86.4%)	262 (74.6%)	p = 0.1098
Non-Hematology	94 (24.2%)	5 (13.5%)	89 (25.4%)	(Chisquare)
Current Smoker				
0	297 (77.3%)	26 (72.2%)	271 (77.9%)	p = 0.4406
1	87 (22.7%)	10 (27.8%)	77 (22.1%)	(Chisquare)
Former Smoker				
0	191 (63.7%)	17 (60.7%)	174 (64.0%)	p = 0.7330
1	109 (36.3%)	11 (39.3%)	98 (36.0%)	(Chisquare)
Arterial Clot				
Y	19 (4.9%)	1 (2.7%)	18 (5.1%)	p = 1.000
N	369 (95.1%)	36 (97.3%)	333 (94.5%)	(Fisher's Exact)
Erythrocytosis HCT: Males				
Y		3 (20.0%)	98 (51.3%)	**0.0195**

N	101 (49.0%)	12 (80.0%)	93 (48.7%)	(Chisquare)
Erythrocytosis HCT:	105 (51.0%)			
Females				
Y		13 (59.1%)	58 (36.5%)	**0.0417**
N		9 (40.9%)	101 (63.5%)	(Chisquare)
	71 (39.2%)			
	110 (60.8%)			
Erythrocytosis HGB: Males				
Y	67 (32.5%)	0 (0%)	67 (35.1%)	**0.0031**
N	139 (67.5%)	15 (100%)	124 (64.9%)	(Fisher's Exact)
Erythrocytosis HGB:				
Females				
Y		9 (40.9%)	43 (27.0%)	0.1780
N	52 (28.7%)	13 (59.1%)	116 (73.0%)	(Chisquare)
	129 (71.3%)			
Leukocytosis				
Y	85 (21.9%)	16 (43.2%)	69 (19.7%)	**0.0010**
N	303 (78.1%)	21 (56.8%)	282 (80.3%)	(Chisquare)
Mesenteric Clot				
Y	33 (8.5%)	4 (10.8%)	29 (8.3%)	0.5402
N	354 (91.5%)	33 (89.2%)	321 (91.7%)	(Fisher's Exact)
Thrombocytosis				
Y	88 (22.7%)	24 (64.9%)	64 (18.2%)	**< 0.0001**
N	300 (77.3%)	13 (35.1%)	287 (81.8%)	(Chisquare)
Venous Clot				

Y	44 (11.3%)	4 (10.8%)	40 (11.4%)	1.000
N	344 (88.7%)	33 (89.2%)	311 (88.6%)	(Fisher's Exact)

Table 2. Ten Best Youden's J scores for LN(EPO) Cutoff.

LN(EPO) Cutoff	EPO Cutoff	Sensitivity	Specificity	Youden's J
1.79182	**6.000363196**	**0.6875**	**0.83333**	**0.52083**
1.94597	7.000418969	0.6875	0.75362	0.44112
1.38633	4.000142558	0.5	0.89855	0.39855
2.07951	8.000547685	0.75	0.64493	0.39493
1.09864	3.000083135	0.4375	0.94928	0.38678
1.60949	5.000260445	0.5	0.86957	0.36957
2.1973	9.00067883	0.75	0.55797	0.30797
2.30267	10.00084911	0.75	0.52174	0.27174
0.69316	2.000025639	0.25	0.98551	0.23551
2.485	12.00112025	0.8125	0.4058	0.2183

Table 3. Summarizing the ROC Curve AUC measures and suggested cutpoints for all continuous blood count measures. If the AUC is not significant, determination of a cutoff value is not advised.

Variable	AUC (95% CI)	AUC p-value	Cutoff	Youden's J	Sensitivity	Specificity
LN(EPO)	**0.7355 (0.5631, 0.9080)**	**0.0074**	LN(EPO) = 1.79 EPO = 6.00	0.52083	0.6875	0.8333
WBC	**0.6988 (0.6119, 0.7857)**	**<0.0001**	8.6043	0.35809	0.81081	0.54728
Hb	**0.6161 (0.5252, 0.7070)**	**0.0123**	17.1043	0.20297	0.86486	0.33811
Hct	0.5565 (0.4545, 0.6584)	0.2776	AUC not significant: No Cutoff determined			
LN(Plts)	**0.7401 (0.6290, 0.8512)**	**<0.0001**	LN(Plts) = 6.0914 Plts = 442	0.50252	0.64865	0.85387
LN(Cr)	0.5602 (0.4481, 0.6723)	0.2923	AUC not significant: No Cutoff determined	0.52083		0.8333

Table 4. Univariate Logistic Regression results with EPO as independent variables of interest. For HCT and HGB, sex is also included in the (multivariate) logistic regression model. OR = Odds of EPO among those with a condition vs Odds of EPO value among those without. For categorical EPO (EPO <= 6 vs. EPO > 6), it is odds of low EPO among those with a condition vs. odds of low EPO among those without a condition.

Model	Odds Ratio (95% CI)	p-value
LFTs = EPO (continuous)	1.002 (0.999, 1.004)	0.2134
LFTs = EPO (categorical)	1.313 (0.799, 2.155)	0.2826
Thrombosis_Hx = EPO (continuous)	1.000 (0.998, 1.002)	0.7031
Thrombosis_Hx = EPO (categorical)	**2.217 (1.336, 3.677)**	**0.0021**
CVD = EPO (continous)	0.997 (0.990, 1.004)	0.3378
CVD = EPO (categorical)	0.687 (0.430, 1.098)	0.1164
Pulmonary Disease = EPO (continuous)	0.998 (0.993, 1.002)	0.3423
Pulmonary Disease = EPO (categorical)	0.742 (0.474, 1.161)	0.1915
O2 = EPO (continuous)	0.996 (0.983, 1.010)	0.5952
O2 = EPO (categorical)	0.637 (0.336, 1.209)	0.1679
Arterial Clot = EPO (continuous)	0.997 (0.979, 1.016)	0.7638
Arterial Clot = EPO (categorical)	1.268 (0.446, 3.603)	0.6563
Erythro HCT = EPO (continuous) + sex + EPO*sex		
EPO (M):	0.998 (0.994, 1.002)	0.1382
EPO (F)	0.983 (0.960, 1.006)	0.1382
Erythro HCT = EPO (categorical) + sex + EPO*sex		
EPO (M):	**0.306 (0.169, 0.555)**	**< 0.0001**
EPO (F)	**0.229 (0.111, 0.470)**	**< 0.0001**
Erythro HGB = EPO (continuous) + sex + EPO*sex		
EPO (M):	0.999 (0.996, 1.002)	0.0824
EPO (F)	0.939 (0.873, 1.009)	0.0824

Erythro HGB = EPO (categorical) + sex + EPO*sex		
EPO (M):	**0.420 (0.231, 0.766)**	**0.0007**
EPO (F)	**0.477 (0.233, 0.975)**	**0.0007**
Leukocytosis = EPO (continuous)	1.000 (0.998, 1.003)	0.6580
Leukocytosis = EPO (categorical)	**2.254 (1.245, 4.079)**	**0.0073**
Mesenteric Clot = EPO (continuous)	0.871 (0.680, 1.115)	0.2736
Mesenteric Clot = EPO (categorical)	**15.999 (2.160, 118.514)**	**0.0067**
Thrombocytosis = EPO (continuous)	**0.893 (0.812, 0.982)**	**0.0195**
Thrombocytosis = EPO (categorical)	**6.992 (3.121, 15.666)**	**< 0.0001**
Venous Clot = EPO (continuous)	0.994 (0.968, 1.021)	0.6676
Venous Clot = EPO (categorical)	2.172 (0.977, 4.828)	0.0569

Figures

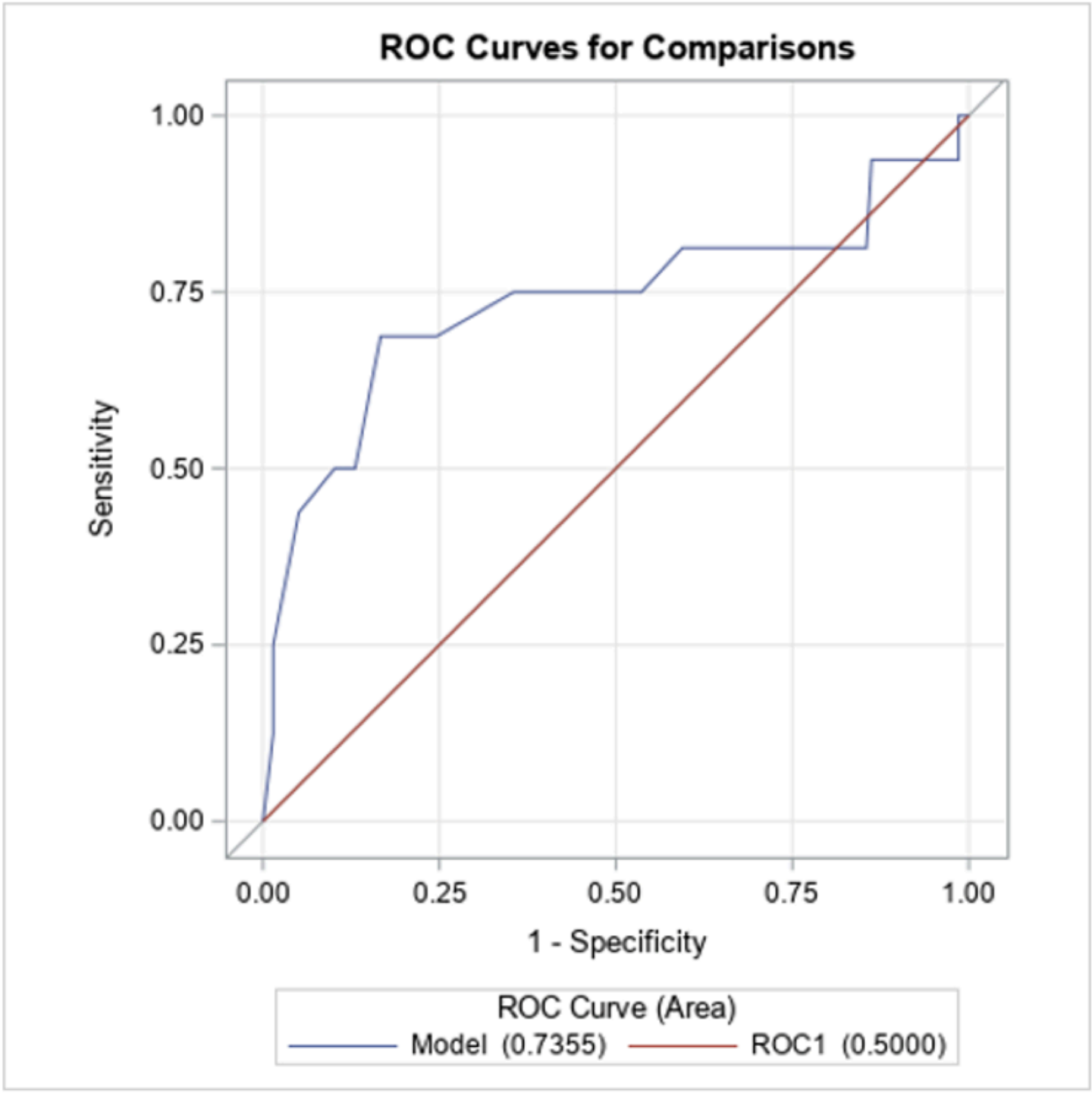


Figure 1

ROC Curve for ln(EPO) status and JAK2 status.

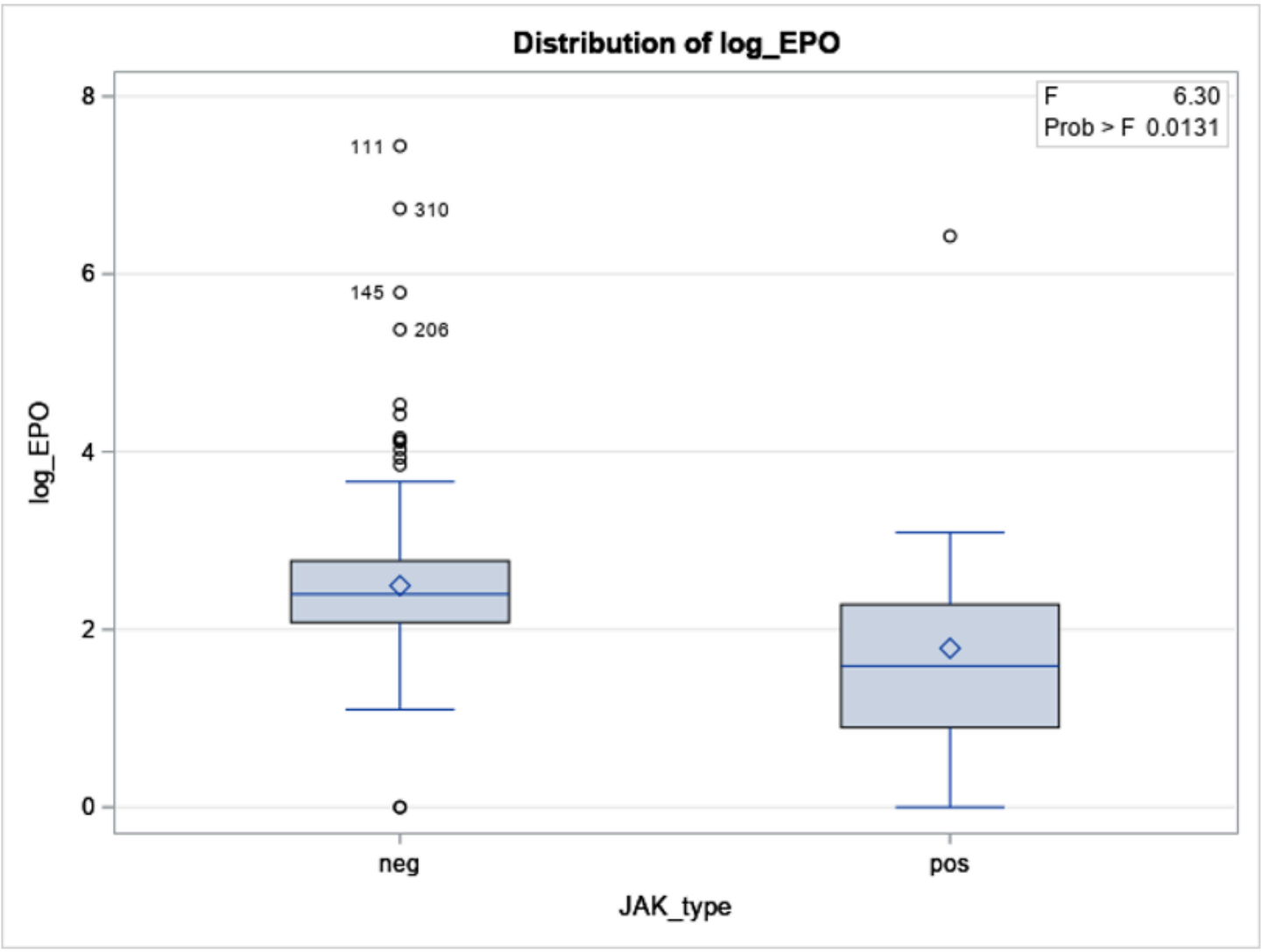


Figure 2

Distribution of LN(EPO) values by JAK2 Status

JAK2 Positive: Mean LN(EPO) = 1.79; Minimum = 0; Maximum = 6.43; Standard Deviation = 1.55

JAK2 Negative: Mean LN(EPO) = 2.49; Minimum = 0; Maximum = 7.44; Standard Deviation = 1.00