

# Evaluation of Hemoglobin and Serum Erythropoietin Levels in Patients with Polycythemia Vera and Secondary Polycythemia

Alireza Khorshid, Daniel González, Jianzhi Zhang

Department of Clinical Laboratory Science, School of Health Professions, University of Texas Medical Branch, Galveston, USA  
Email: Ali.r.khorshid@gmail.com, dagonza1@utmb.edu, jzzhang@utmb.edu

**How to cite this paper:** Khorshid, A., González, D. and Zhang, J.Z. (2024) Evaluation of Hemoglobin and Serum Erythropoietin Levels in Patients with Polycythemia Vera and Secondary Polycythemia. *Journal of Biosciences and Medicines*, 12, 51-59.

<https://doi.org/10.4236/jbm.2024.128005>

**Received:** June 28, 2024

**Accepted:** August 3, 2024

**Published:** August 6, 2024

Copyright © 2024 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

## Abstract

**Objective:** This study compares hemoglobin and erythropoietin levels in patients with polycythemia vera and secondary polycythemia. **Study Design:** A retrospective cross-sectional study evaluating the serum erythropoietin and hemoglobin levels in patients with polycythemia vera and secondary polycythemia. This study was performed simultaneously in Texas state of the U.S. and Fars Province in Iran. **Methods:** Hemoglobin, hematocrit and erythropoietin test results were collected from patients aged 19 to 75 years who were diagnosed with polycythemia vera and secondary polycythemia. Patients records with history of thrombocytopenia, congestive heart failure, dyspnea, anemia and pregnant woman were excluded from study. Patients in each decade of life were examined in separate groups, so that changes in hemoglobin related to aging did not affect the research results. **Results:** 75% of the patients were men, and 25% were women. A total of 1580 patients were analyzed in this study. 57.3% of patients in UTMB and 38.8 patients in Iran have hemoglobin level above 17 mg/dl. 74% of patients in UTMB and 88% of patients in Iran have erythropoietin below 10 IU/mL. Polycythemia in UTMB was more common in people over 50 and in Iran in patients under 50 years old. The serum hemoglobin and erythropoietin levels in patients with polycythemia vera were not significantly different in compare to secondary polycythemia patients. Data showed that there were 84 polycythemia patients per 100,000 people. The results of this study in UTMB and Iran showed that 4.5% and 7%, respectively, of patients with polycythemia had a positive JAK2 test. **Conclusion:** Low erythropoietin levels may not be helpful in differentiating polycythemia vera from secondary polycythemia.

---

## Keywords

Polycythemia Vera, Erythropoietin, JAK2, Secondary Polycythemia

---

### 1. Introduction

Polycythemia is an excessive increase in the number of red blood cells that increases the viscosity of blood in arteries [1]. There are two types of polycythemia, polycythemia vera or primary polycythemia, and second types of polycythemia occur in response to hypoxia or increasing erythropoietin levels [1]. Polycythemia vera has similar clinical symptoms to secondary polycythemia [1].

The diagnosis and treatment of Polycythemia vera via molecular genetic test and detection of a mutation in Janus kinase 2 (JAK2) has made progress over the last two decades [2]. Patients with secondary polycythemia have high hemoglobin levels, but no mutations in JAK2 genes are detected in these patients [3]. According to the World Health Organization (WHO), the diagnosis of myeloproliferative neoplasm (MPN) is based on cell count, peripheral blood smear, bone marrow examination, and molecular genetic tests [4].

JAK2 is a protein with tyrosine kinase properties and belongs to the intracellular Janus kinase family. Somatic mutations in this protein were observed in different types of myeloproliferative neoplasms [5]. Erythropoietin (EPO) is a hormone produced primarily by the kidneys. Erythropoietin plays a key role in the production of red blood cells, which are produced and released into the blood in response to hypoxemia [6]. In polycythemia vera, the level of erythropoietin in the blood decreases. So the body uses a dynamic feedback system to help maintain sufficient oxygen levels and a relatively stable number of RBCs in the blood [6].

The purpose of this study was to evaluate the serum erythropoietin level and hemoglobin level in polycythemia vera and secondary polycythemia to evaluate the ability of these two tests to differentiate these two diseases. In patients with JAK2-negative test results, no invasive tests are needed if routine laboratory tests such as hemoglobin and serum erythropoietin can predict polycythemia vera.

Polycythemia vera is usually asymptomatic, progresses very slowly and usually occurs over the years. For this reason, many patients are diagnosed via routine laboratory tests before the patient becomes symptomatic, including itchy skin or dizziness [7]. In addition to all the nonspecific symptoms in some patients, itching of the skin after bathing can help in diagnosis.

According to the 2016 edition of the WHO guidelines for the diagnosis of polycythemia vera [8], diagnosis requires the presence of one of three major criteria or the first two major criteria and the minor criterion, as shown in (Table 1).

**Table 1.** WHO guidelines for the diagnosis of polycythemia vera.

<b>Major Criteria</b>
1. Hb > 16.5 g/dL or HCT > 49% in men Hb > 16 or HCT > 48% in women
2. B. M biopsy showing hypercellularity
3. JAK2 exon 14 V617F or exon 12 mutation
<b>Minor Criterion</b>
Subnormal Serum Erythropoietin level

Note: Diagnosis of PV requires meeting either all 3 major criteria or the first 2 major criteria and minor criteria.

Patients with high hemoglobin and low erythropoietin levels should have either a JAK2 mutation or a bone marrow examination to be able to be diagnosed with polycythemia vera. Therefore, in patients with JAK2 mutation-negative test results, the only way to diagnose the disease is to perform a bone marrow examination. JAK2 V617F is the most common mutation in polycythemia vera. Therefore, in some cases, diagnosing these two diseases without consideration of bone marrow morphology is difficult [9].

## 2. Methods

A retrospective cross-sectional study. Test results from the last 3 years were collected from three laboratories in Fars Province, Iran, which were licensed in the field of molecular genetics. These results were collected from patients who were referred to the laboratory for the JAK2 test. These results included JAK2, CBC and erythropoietin test results. The results of the same tests were also collected from the laboratories of UTMB-affiliated hospitals in Galveston City, Texas State, of the U.S. The sample size in this study was determined to be 360, so that each group of laboratories had a sample size of 180. The power analysis was calculated with a confidence level of 95% and a desired power of 0.80.

The Iranian laboratories were limited to Fars Province, which is in the southern part of the country. The data collected from Texas was limited to the University of Texas Medical Branch Affiliation Hospital using the TriNetX health research network website. Using online system, records from patients who had been diagnosed with polycythemia vera early in the past 3 years were collected. Clinical data and test results for these patients were extracted from the TriNetX system. The variables used in this study included age and sex and the results of hemoglobin, hematocrit, erythropoietin and JAK2 tests. In the analysis of the results, patients in each decade of life were examined in separate groups, so that changes in hemoglobin related to aging did not affect the research results.

### Exclusion criteria

Patients with high platelet count were excluded from the study due to the possibility of developing essential thrombocythemia. Patients under 19 and over 75

years of age were excluded from study because their reference ranges of hemoglobin and erythropoietin are different. Patients with history of congestive heart failure and Dyspnea were excluded from the study because of abnormal hemoglobin levels. Also, data from pregnant women and patients with low Ferritin levels were excluded from study because of decrease in hemoglobin levels.

### 3. Results

#### Analysis of Data Collected from Texas

The data were extracted using TriNetX from University of Texas Medical Branch affiliation hospitals. 1600 patients were diagnosed with polycythemia. Of these, 1590 patients were older than 19 years. A total of 1290 patients aged between 19 and 75 years were included. The target population of the study included 750 patients in the last 3 years, 590 patients in the last 2 years and 390 patients in the last 12 months.

#### Statistical population of polycythemia patients in the UTMB cohort

A total of 75% (967) of the patients were men, and 25% (323) were women. A total of 1580 patients were analyzed in this study. The mean age of the patients was 57 years. On average, 86% of patients with polycythemia are between 19 and 75 years of age each year.

It can be concluded that 31% of patients are under 50 years old, the highest prevalence of polycythemia was in the age range of 61 to 70 years, and the risk of polycythemia disease increases with age (**Table 2**). The risk of developing polycythemia in the third and fourth decades of life is less than 10%.

**Table 2.** Percentages of patients in different age groups in the UTMB.

Date		Age groups					
From	To	19 - 30	31 - 40	41 - 50	51 - 60	61 - 70	71 - 75
Jan. 1 2016	Jun. 1 2021	6.4%	9.1%	16.1%	24.7%	30.1%	13.4%

#### Analysis of Hemoglobin, Erythropoietin and JAK2 levels in UTMB patients

The mean hemoglobin and hematocrit concentrations in male patients were 17.3 mg/dl and 51.5%, respectively. The mean hemoglobin and hematocrit concentrations in female patients were 16.1 mg/dl and 50.2%, respectively (**Table 3**).

**Table 3.** Number of hemoglobin tests based on test results in different ranges.

	Hemoglobin concentration						
	Hb < 15	15 - 15.9	16 - 16.9	17 - 17.9	18 - 18.9	19 - 19.9	Hb > 20
Number of patients	105	96	115	211	115	67	38
Percent	14%	12.8%	15.3%	28.1%	15.3%	8.9%	5.0%

Comparing hemoglobin level of patients with polycythemia vera and secondary polycythemia, the f-ratio was 2.55, and the p-value was 0.11. thus, the result was not significant at  $p < 0.05$ . Moreover, there was no significant difference in the hemoglobin level between polycythemia vera and secondary polycythemia in UTMB patients. There were only 15 polycythemia vera patients at UTMB over the past 3 years (aged between 19 and 75 years) (**Table 4**).

**Table 4.** Number of polycythemia patients based on different ranges of erythropoietin test results at the UTMB.

	EPO level			
	EPO < 5	6 < EPO < 10	11 < EPO < 20	EPO > 21
Number of patients	25	35	15	5
Percent	31%	43%	18%	6%

Comparing the erythropoietin levels of patients with polycythemia vera and secondary polycythemia, The F-ratio was 3.350, and the p-value was 0.07; thus, the result was not significant at  $p < 0.05$ . There was no significant difference in erythropoietin levels between polycythemia vera and secondary polycythemia in UTMB patients.

#### Analysis of Data Collected from Iran

##### Population of polycythemia vera in Iran

Of the 370 patient records collected from Iranian laboratories, 356 were in the age range of 19 to 75 years and were analyzed in this study. A total of 242 males (68.2%) and 114 females (31.8%) were included (**Table 5**). The data in this table show that the highest incidence of polycythemia occurred in the third and fourth decades of life. **Table 5** shows that the incidence of polycythemia was greater in the third decade of life and decreases with increasing age.

**Table 5.** Percentage of polycythemia patients in different age groups in Iran.

Date		Age groups					
From	To	19 - 30	31 - 40	41 - 50	51 - 60	61 - 70	71 - 75
Jan. 1 2018	Jun. 1 2021	32.8%	25.2%	21.3%	12.0%	9.2%	3.6%

##### Analysis of Hemoglobin, Erythropoietin, and JAK2 Levels in Iran

This information showed that hemoglobin 16 to 16.9 was more common among polycythemia patients. A total of 242 male patients were included in this study. The mean hemoglobin level in male patients was 16.9 mg/dl, and the mean hematocrit in male patients was 50.8% (**Table 6**). A total of 114 female polycythemia patients ranging in age from 19 to 75 years were included; the mean hemoglobin level was 15.1 mg/dl, and the mean hematocrit level was 46.0%.

**Table 6.** Number of hemoglobin tests based on test results in different ranges (males and females).

	Hemoglobin level						
	Hb < 15	15 - 15.9	16 - 16.9	17 - 17.9	18 - 18.9	19 - 19.9	Hb > 20
Number of patients	43	66	78	52	43	31	8
Percent	17%	20.3%	23.5%	15.4%	12.1%	8.9%	2.4%

Comparing the hemoglobin levels of patients with polycythemia vera and secondary polycythemia, the f-ratio was 1.05, and the p-value was 0.30; thus, the result was not significant at  $p < 0.05$ . There was no significant difference in hemoglobin levels between Iranian male patients with polycythemia vera in compare to secondary polycythemia patients. A total of 305 JAK2 test results were collected from patients with polycythemia; 14 tests were positive, and 291 were negative. A total of 4.5% of patients had a positive JAK2 test result. There were 20 polycythemia vera-diagnosed patients aged 19 to 75 years in records collected from Iran.

Comparing the erythropoietin levels of patients with polycythemia vera and secondary polycythemia (**Table 7**). The F-ratio was 0.50, and the p-value was 0.48; thus, the result was not significant at  $p < 0.05$ . There was no significant difference in erythropoietin levels between polycythemia vera patients and secondary polycythemia patients in Iran.

**Table 7.** Number of patients with different erythropoietin test results in Iran.

	EPO level			
	EPO < 5	6 < EPO < 10	11 < EPO < 20	EPO > 21
Number of patients	206	91	33	9
Percent	61%	27%	10%	1%

## 4. Discussion

Polycythemia vera is a disorder of hematopoietic stem cells that causes an abnormal increase in red blood cell counts and an increase in red blood cell mass [10]. However, the detection of JAK2 mutations accelerates the diagnostic process in many patients. However, the diagnosis of these patients is still controversial. The results of this study in UTMB and Iran showed that 4.5% and 7%, respectively, of patients with polycythemia had a positive JAK2 test.

This study showed that the incidence of polycythemia vera in UTMB patients was between 50 and 70 years, which was also reported in other studies [11]. An epidemiological study of myeloproliferative disorders reported that 44 to 57 patients per 100,000 people in the U.S. had polycythemia vera [12]. However, the data from this study showed that the prevalence of polycythemia in patients with UTMB is slightly greater. The results extracted from the TriNetX data showed

that there were 84 polycythemia patients per 100,000 people. The statistical population collected in Iran showed that the incidence of this disease was greater in young patients in Iran, so the highest incidence of polycythemia vera in Iran was in the third and fourth decades of life, and most of the statistical population collected from Iran was under the age of 50.

A comparison of hemoglobin levels among patients with polycythemia vera revealed that there was no significant difference in hemoglobin levels between patients with JAK2-positive mutations and patients with JAK2-negative mutations. That was understood that polycythemia vera patients with a negative JAK2 test result have as high hemoglobin as patients with a positive JAK2 test result. The mean hemoglobin levels of male patients in the Iranian and UTMB cohorts were 16.9 and 17.3, respectively. The mean hemoglobin levels of female patients in the Iranian and UTMB cohorts were 15.1 and 16.1, respectively.

Serum erythropoietin levels have been tested in 36% of patients with polycythemia in the UTMB. Subnormal erythropoietin levels was a minor criteria in the diagnosis of polycythemia vera. This study showed that, in the UTMB, only 31% of patients had erythropoietin concentrations less than 5 IU/mL, and 43% of patients had erythropoietin concentrations between 5 and 10. Results obtained from Iran showed that 61% of patients had an erythropoietin level less than 5, and 27% of patients had an erythropoietin level between 5 and 10 IU/mL. The mean erythropoietin levels for polycythemia vera patients in Iranian and UTMB patients were 6.5 and 8.4, respectively. These findings indicate that most patients with polycythemia vera have an erythropoietin concentration of less than 10 IU/mL. A comparison of erythropoietin levels among patients with polycythemia vera revealed no significant difference in erythropoietin levels between patients with JAK2-positive mutations and patients with JAK2-negative mutations. Analysis of the erythropoietin concentration in UTMB patients with a negative JAK2 test revealed that the erythropoietin concentration was lower than patients with a positive JAK2 test. The results obtained from Iran and the UTMB were analyzed separately, but these findings held true for both groups.

According to the guidelines of the World Health Organization for the diagnosis of polycythemia vera, the measurement of erythropoietin was considered a minor criterion. According to the same instructions, bone marrow examination was considered a major criterion.

## 5. Conclusion

The erythropoietin test cannot play a role in diagnosing patients with polycythemia vera. The results of the analysis of UTMB and Iran data showed that erythropoietin test can be used in combination with the JAK2 test for the diagnosis of polycythemia vera. The use of erythropoietin tests, which are inexpensive and have shorter turnaround times, can play a role in reducing the cost of diagnosing polycythemia patients. In patients with JAK2 mutation-negative results, a bone marrow examination should be performed to make a definitive di-

agnosis. This study showed that high hemoglobin and low serum erythropoietin levels in patients without perfortming JAK2 mutations cannot be used to diagnose polycythemia vera. Erythropoietin is measured in laboratories with different techniques such as ELISA, ELFI and chemiluminescence. Differences in the accuracy and precision of laboratory methods can make the results of different techniques not comparable. Erythropoietin measurement is not included in the daily schedule in most laboratories, so erythropoietin testing is done one or two days a week. The quality of storage of patient samples before testing can affect the quality of the results. Polycythemia in most patients were without clinical symptoms or the person has had these symptoms for a long time and were used to it. Because of that, many patients are still undiagnosed.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

### References

- [1] Nguyen, E., Harnois, M., Busque, L., Sirhan, S., Assouline, S., Chamaki, I., *et al.* (2021) Phenotypical Differences and Thrombosis Rates in Secondary Erythrocytosis versus Polycythemia Vera. *Blood Cancer Journal*, **11**, Article No. 75. <https://doi.org/10.1038/s41408-021-00463-x>
- [2] Buyukasik, Y., Al, I.R., Ar, C., Turgut, M., Yavuz, S. and Saydam, G. (2018) Polycythemia Vera: Diagnosis, Clinical Course, and Current Management. *Turkish Journal of Medical Sciences*, **48**, 698-710.
- [3] McMullin, M.F.F., Mead, A.J., Ali, S., Cargo, C., Chen, F., Ewing, J., *et al.* (2018) A Guideline for the Management of Specific Situations in Polycythaemia Vera and Secondary Erythrocytosis: A British Society for Hematology Guideline. *British Journal of Haematology*, **184**, 161-175. <https://doi.org/10.1111/bjh.15647>
- [4] Kvasnicka, H.M. (2019) The Differential Diagnosis of Classical Myeloproliferative Neoplasms (MPN): The Updated WHO Criteria. *Rinsho Ketsueki—Japanese Journal of Clinical Hematology*, **60**, 1166-1175.
- [5] Tefferi, A., Lavu, S., Mudireddy, M., Lasho, T.L., Finke, C.M., Gangat, N., *et al.* (2018) *JAK2* Exon 12 Mutated Polycythemia Vera: Mayo-Careggi MPN Alliance Study of 33 Consecutive Cases and Comparison with *JAK2* V617F Mutated Disease. *American Journal of Hematology*, **93**, E93-E96. <https://doi.org/10.1002/ajh.25017>
- [6] Mossuz, P., Girodon, F., Donnard, M., Latger-Cannard, V., Dobo, I., Boiret, N., Le-cron, J.C., Binquet, C., Barro, C., Hermouet, S. and Praloran, V. (2004) Diagnostic Value of Serum Erythropoietin Level in Patients with Absolute Erythrocytosis. *Hematologica*, **89**, 1194-1198.
- [7] Ancochea, À., Álvarez-Larrán, A., Morales-Indiano, C., García-Pallarols, F., Martínez-Avilés, L., Angona, A., *et al.* (2014) The Role of Serum Erythropoietin Level and *JAK2* V617f Allele Burden in the Diagnosis of Polycythaemia Vera. *British Journal of Haematology*, **167**, 411-417. <https://doi.org/10.1111/bjh.13047>
- [8] Arber, D.A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M.J., Le Beau, M.M., *et al.* (2016) The 2016 Revision to the World Health Organization Classification of Myeloid Neoplasms and Acute Leukemia. *Blood*, **127**, 2391-2405. <https://doi.org/10.1182/blood-2016-03-643544>

- 
- [9] Tefferi, A. and Barbui, T. (2018) Polycythemia Vera and Essential Thrombocythemia: 2019 Update on Diagnosis, Risk-Stratification and Management. *American Journal of Hematology*, **94**, 133-143. <https://doi.org/10.1002/ajh.25303>
- [10] Spivak, J.L. (2017) Myeloproliferative Neoplasms. *New England Journal of Medicine*, **376**, 2168-2181. <https://doi.org/10.1056/nejmra1406186>
- [11] Griesshammer, M., Gisslinger, H. and Mesa, R. (2015) Current and Future Treatment Options for Polycythemia Vera. *Annals of Hematology*, **94**, 901-910. <https://doi.org/10.1007/s00277-015-2357-4>
- [12] Mehta, J., Wang, H., Iqbal, S.U. and Mesa, R. (2013) Epidemiology of Myeloproliferative Neoplasms in the United States. *Leukemia & Lymphoma*, **55**, 595-600. <https://doi.org/10.3109/10428194.2013.813500>

## Abbreviations

JAK2: Janus Activator Kinase 2.