Changes in neutrophil-to-lymphocyte ratio in patients with polycythemia vera treated with ruxolitinib reflect *JAK2* variant allele frequency

The neutrophil-to-lymphocyte ratio (NLR) has received interest as a potential biomarker of diseases in which sustained inflammation plays a major role, including cardiovascular diseases and tumors.[1] In cancer, tumor growth and dissemination are facilitated by chronic inflammation promoted by activated neutrophils, while systemic and tumor-infiltrating T-lymphocytes are supposed to counteract cancer development by activating immunosurveillance. Similar processes may occur in the vascular system, resulting in atherosclerosis and vascular occlusion. Capturing the balance between factors promoting and inhibiting cancer and atherosclerosis, NLR may serve as a simple and reliable prognostic biomarker of such different diseases.[1]

In polycythemia vera (PV), a myeloproliferative neoplasm in which a sustained inflammatory condition and abnormal neutrophil count and functions are fueled by uncontrolled activation of the JAK/STAT pathway caused by a *JAK2*^{V617F} mutation, higher NLR was associated with risk of venous thrombosis,[2] reduced survival,[3] and response to ropeg-interferon.[4] In a Danish population-based longitudinal analysis, increasing NLR was associated with shorter survival in patients with PV and other myeloproliferative neoplasms, compared to survival of the unaffected population.[5] A comparative analysis of PV patients treated with hydroxyurea or phlebotomy (ECLAP study, N=202 each), ropeg-interferon or phlebotomy (LOW-PV trial, N=63 and N=64, respectively), and ropeg-interferon or hydroxyurea (PROUD-PV/CONTINUATION-PV study, N=127 each) showed unique kinetic patterns of NLR upon different treatments. In fact, NLR resulted unchanged from baseline in patients managed with phlebotomy only and hydroxyurea, in the latter instance because of a parallel decline of neutrophils and lymphocytes, while in ropeg-interferon-treated patients, the reduction of NLR was due to preferential lowering of neutrophils.[6] Recent data indicate that neutrophils in PV express reduced levels of Kruppel-like factor 2, in a manner inverse to the *JAK2*^{V617F} variant allele frequency (VAF), which were largely reverted by pegylated interferon alfa.[7] Furthermore, tissue factor synthesis and activity was described in PV neutrophils, possibly contributing to thrombotic risk.[8] An additional explanation for abnormally increased NLR levels and the differential effects of drugs may be related to expression of the *JAK2*^{V617F} mutation also in the lymphoid lineage, which in a preclinical model caused impaired lymphoid differentiation.[9] The reduction of NLR in patients receiving ropeg-interferon correlated significantly with decrease of *JAK2*^{V617F} VAF, and molecular responses to ropeg-interferon were predictive of improved event-free survival.[6,10] Also relevant to the interpretation of these findings is the evidence, obtained in animals expressing ruxolitinib-resistant mutations, that the action of ruxolitinib was independent of *JAK2*^{V617F} signaling, rather it appeared to be mediated by reduction of pro-inflammatory cytokines produced by stromal cells and non-malignant hematopoietic cells.[11] Another drug currently used for second-line treatment of PV patients is ruxolitinib, a JAK1 and JAK2 inhibitor.[12,13] Recent data from the MAJIC study and a real-world cohort have shown that the progressive decline of *JAK2*^{V617F} VAF under ruxolitinib treatment correlated with improved overall and event-free survival, particularly myelofibrosis-free survival.[14,15]

The aims of the current study were: (i) to analyze time-dependent changes of NLR, total leukocyte count, and absolute counts of neutrophils and lymphocytes in a prospective cohort of patients with PV who received ruxolitinib as second-line therapy, and (ii) to exploit a correlation of changes, between baseline and upon treatment, in *JAK2*^{V617F} VAF and NLR levels and their relationships with disease characteristics. The study included 41 patients with PV who received ruxolitinib, 10 mg BID for a median of 8.7 years (range, 0.8-9.8) because of intolerance (44.2%) or resistance (55.8%) to hydroxyurea; the overall follow-up was 9.2 years (range, 1.3-14.5). Patients were managed at our Institution according to conventional treatment criteria for PV and following the label of ruxolitinib (10 mg BID) as part of an observational study that allowed serial samples to be prospectively collected for exploratory analyses. The study was approved by an Ethics Committee as part of the MYNERVA project (#21267; MYeloid NEoplasms Research Venture AIRC); participants provided signed inform consent. The cohort was enriched in patients with advanced disease, as shown by the long disease duration prior to ruxolitinib treatment (median, 6.2 years) and the high *JAK2*^{V617F} VAF (median, 68.3%), with 76% of the patients having greater than 50% VAF, which may have contributed to the relatively high rate of evolution to post-PV myelofibrosis (Table 1). Ten patients (24.4%) had a history of thrombosis prior to treatment. All but two patients were still on treatment at the latest timepoint (4 years). A complete clinical-hematologic response and a molecular response, according to the International Working Group-Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) criteria, were observed in 32 (78%) and 17 (41.5%) patients, respectively. The median NLR value in the whole population at baseline

was 5.2 (95% confidence interval [95% CI]: 1.6-25.0); we accordingly divided patients into high (H-, above the median) and low (L-, below the median) NLR categories. Analysis of the patients' characteristics at baseline showed that, when compared to L-NLR patients, those in the H-NLR category had a significantly higher median white blood cell count (12.6 *vs*. 8.1x10^9/L; *P*=0.002), due to increased absolute neutrophil count (12.1 *vs.* 5.9x10^9/L; *P*<0.001) while absolute lymphocyte count (1.4 *vs*. 2.2x10^9/L; *P*=0.001) was lower. Conversely, hemoglobin level (15.0 *vs*. 14.0 g/dL; *P*=0.01) and platelet count (541 *vs*. 323.5x10^9/L; *P*=0.006) were higher in L-NLR compared to H-NLR patients. There was no baseline difference in disease duration, splenomegaly, evolution to post-PV myelofibrosis and cardiovascular events between the two categories of patients (Table 1).

In the entire cohort, NLR value at baseline resulted correlated with *JAK2*^{V617F} VAF (r=0.5, *P*=0.001; Pearson test). Accordingly, *JAK2*^{V617F} VAF (mean ± standard deviation [SD]) was significantly higher in H-NLR patients (81.8±13.1%) than in L-NLR ones (54.8±23.8%; *P*<0.001), while the proportion of patients with VAF ≥50% was 100% *versus* 52.4% (*P*<0.001), respectively. Furthermore, the correlation between NLR ratio and *JAK2*^{V617F} VAF was maintained during ruxolitinib treatment, with correlation coefficients of 0.60 (*P*=0.004) at 2 years, 0.55 (*P*=0.048) at 3 years, and 0.66 (*P*=0.003) at 4 years. However, after starting ruxolitinib, the changes in NLR observed in the two categories differed. In H-NLR patients, the median baseline NLR value of 9.6 (95% CI: 5.3-25.0) decreased to 5.4 (95% CI: 2.7-11.0; *P*=0.002), 5.2 (95% CI: 1.9-8.7; *P*=0.001) and 4.2 (95% CI: 1.3-11.5; *P*=0.001) at 1, 2, and 3 years, remaining steadily stable afterwards (Figure 1A). Changes in NLR levels were due to progressive, preferential decrease of absolute neutrophil counts (from a median of 12.1x10^9/L at baseline to 6.7x10^9/L (-45%; *P*=0.001), 6.4x10^9/L (-47%; *P*=0.002), and 5.0x10^9/L (-59%; *P*=0.001) at 1, 2, and 3 years), while absolute lymphocyte counts

Table 1. Main clinical characteristics at baseline and outcome of patients divided according to high or low neutrophil-to-lymphocyte ratio.

Variables	All patients N=41	High NLR N=20	Low NLR N=21	*P*
Age, years, median (range)	56.1 (34.6-74.3)	57.9 (34.6-74.3)	55.5 (37.7-71.1)	0.58
Female gender, N (%)	21 (51.2)	10 (50)	11 (52.4)	0.88
Hemoglobin, g/dL, median (range)	14.7 (12.0-18.7)	14.0 (12.0-17.6)	15.0 (12.6-18.7)	0.01
Hematocrit, %, mean (range)	47 (38.0-57.7)	46.2 (38.0-51.0)	47.9 (39.2-57.7)	0.002
Leukocyte count, 10^9/L, median (range)	10.7 (4.8-25.5)	12.6 (6.5-21.6)	8.1 (4.8-25.5)	0.002
ANC, 10^9/L, median (range)	8.8 (9.4-26.3)	12.1 (5.1-26.3)	5.9 (4.0-15.8)	<0.001
ALC, 10^9/L, median (range)	1.6 (0.6-6.5)	1.4 (0.6-2.8)	2.2 (1.0-6.5)	0.001
Platelet count, 10^9/L, median (range)	389 (111-898)	323.5 (111-757)	541 (156-898)	0.006
JAK2^{V617F} VAF, %, mean ± SD	68.3±23.4	81.8±13.1	54.8±23.8	<0.001
Patients with *JAK2*^{V617F} VAF ≥50%, N (%)	31 (75.6)	20 (100)	11 (52.4)	<0.001
Major thrombosis at or prior to diagnosis, N (%)	10 (24.4)	6 (30.0)	4 (19.0)	0.41
Thrombosis score, N (%) Low High	 12 (29.3) 29 (70.7)	 5 (25.0) 15 (75.0)	 7 (33.3) 14 (66.7)	0.56
Palpable splenomegaly, N (%)	30 (73.2)	17 (85.0)	13 (61.9)	0.09
Disease duration before starting ruxolitinib, years, median (range)	6.2 (0.7-22.6)	7.9 (0.7-17.1)	3.83 (0.7-22.6)	0.36
Duration of ruxolitinib therapy, years, median (range)	8.7 (0.8-14.5)	8.6 (0.8-14.5)	8.0 (1.3-14.3)	0.75
Patients achieving CHR, N (%)	32 (78)	16 (80.0)	16 (76.2)	0.77
Patients achieving MR, N (%)	17 (41.5)	7 (35.0)	10 (47.6)	0.41
Thrombosis, N (%)	2 (4.8)	1 (5.0)	1 (4.7)	-
Evolution to post-PV MF, N (%)	16 (39.0)	9 (45.0)	7 (33.3)	0.44
Evolution to blast phase, N (%)	2 (4.9)	1 (5.0)	1 (4.8)	0.97
Deaths, N (%)	3 (7.9)	1 (5.0)	2 (9.5)	0.58

ALC: absolute lymphocyte count; ANC: absolute neutrophil count; CHR: clinical-hematologic response; MF: myelofibrosis; MR: molecular response; NLR: neutrophil-to-lymphocyte ratio; PV: polycythemia vera; SD: standard deviation; VAF: variant allele frequency.

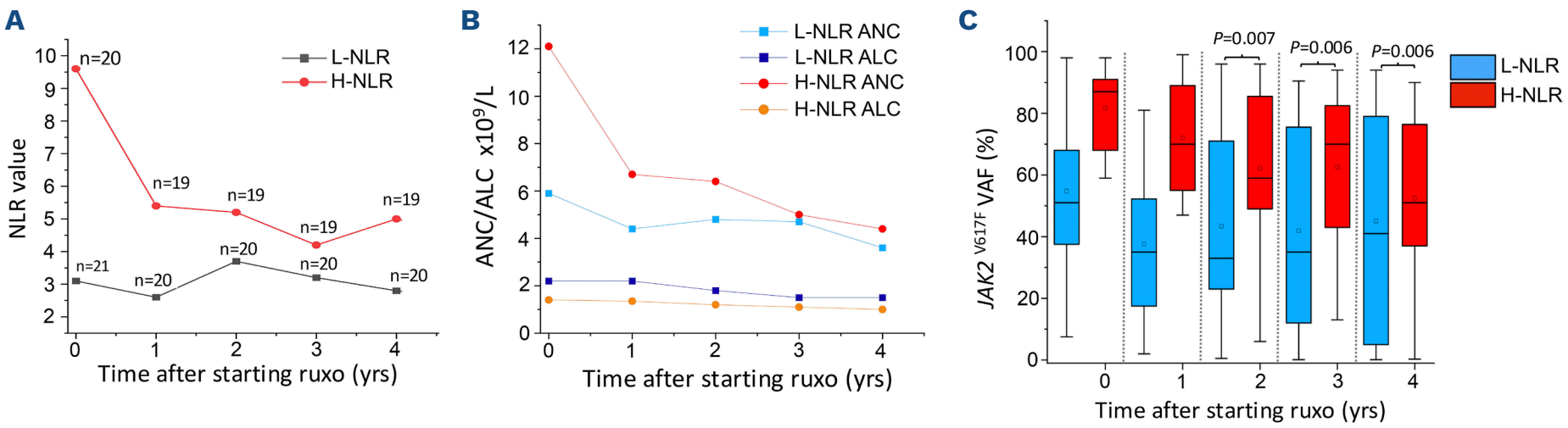


Figure 1. Changes in neutrophil-to-lymphocyte ratio, absolute neutrophil and lymphocyte counts, and *JAK2* variant allele frequency during treatment with ruxolitinib. (A) The kinetics of changes in the values of the neutrophil-to-lymphocyte ratio (NLR) during 4 years of treatment with ruxolitinib in the categories of patients with Low-NLR (L-NLR) or High-NLR (H-NLR), as defined at baseline (see text for definitions). The number of patients available at each timepoint is indicated. (B) The corresponding changes in absolute neutrophil and lymphocyte counts in the two categories of patients. (C) Boxes show the levels of *JAK2*^{V617F} variant allele frequency in the two categories of patients at each annual timepoint. Statically significant differences between L-NLR and H-NLR are shown on the top for each set of data. The time "0" refers to baseline values, before starting ruxolitinib treatment. ALC: absolute lymphocyte count; ANC: absolute neutrophil count; Ruxo: ruxolitinib; VAF: variant allele frequency; yrs: years.

remained largely the same, from a baseline of 1.4x10^9/L (range, 0.1-2.8) to 1.35 (range, 1.0-3.0; *P*=0.5), 1.24 (range, 1.0-3.0; *P*=0.6), and 1.14 (range, 1.0-5.0; *P*=0.4) (Figure 1B). Conversely, in the L-NLR group, no statistically significant modification of NLR was noticed, with values of 3.1x10^9/L (range, 1.6-5.0) at baseline to 2.6 (range, 1.1-8.2; *P*=0.5), 3.0 (range, 1.0-5.0; *P*=0.16), and 3.2 (range, 1.0-7.7; *P*=0.29) at 1, 2, and 3 years, respectively (Figure 1A, B).

Changes in *JAK2*^{V617F} VAF also followed different patterns in the two NLR categories. In the H-NLR category, a baseline VAF value (±SD) of 81.8±13.1% decreased to 72.0±17.6% (*P*=0.07), 62.1±26.0% (*P*=0.007), 62.5±24.3% (*P*=0.006), and 52.5±27.5% (*P*=0.008) at year 1, 2, 3, and 4, respectively. Conversely in the L-NLR group, changes of *JAK2*^{V617F} VAF were not statistically significant at any time point, from a baseline value of 54.8±23.8% to 37.7±24.7% (*P*=0.11), 43.4±30.8% (*P*=0.16), 41.9±34.8% (*P*=0.21), and 45.0±37.4 (*P*=0.36) at year 1, 2, 3, and 4, respectively (Figure 1C).

Baseline NLR levels had no significant relationships with clinical-hematologic response, thrombosis, progression to myelofibrosis, evolution to acute myeloid leukemia, or death during ruxolitinib treatment in either NLR category. However, the decline of NLR at 4 years was associated with a lower rate of progression to post-PV myelofibrosis; patients who did not progress (N=25) had statistically significantly reduced NLR levels (from a median [range] of 4.6 [1.6-21.0] to 2.8 [1.3-7.0], -30% from baseline) compared to those who progressed (N=16; from a median [range] of 5.8 [1.9-13.0] to 4.9 [1.3-8.4], -16%; *P*=0.03). Findings were similar *JAK2*^{V617F} VAF, which decreased from a mean ± SD of 62.6±21.4% to 38.4±18.4% (-39%) in those who did not progress compared to a -11% reduction (from 78.8±16.1% to 63.1±22.6%) in those who did (*P*<0.01).

Overall, these data, with the limitation of small numbers but the value of long follow-up, suggest that higher NLR values in patients with PV are associated with unique clinical characteristics at baseline, including higher *JAK2*^{V617F} VAF, as well as a peculiar response profile in response to ruxolitinib treatment. In fact, H-NLR patients showed statistically significant reductions of NLR values, due to a preferential decrease of ANC, which were associated with significant declines of *JAK2*^{V617F} VAF, unlike in patients with lower baseline NLR values. Of interest, we observed significantly greater reductions of NLR, paralleled by similar declines of *JAK2*^{V617F} VAF, in patients who did not progress to develop post-PV myelofibrosis, compared to those who did develop myelofibrosis. However, we acknowledge that the observed correlation of NLR with *JAK2*^{V617F} VAF reduction and the rate of progression to myelofibrosis do not establish any causal relationship. Given that the current findings are reminiscent of observations in patients with PV treated with ropeg-interferon in the LOW-PV and PROUD-PV/CONTINUATION-PV studies, we propose that monitoring NLR might represent a simple and affordable surrogate test, rather than molecular monitoring, to predict changes of *JAK2* VAF in response to treatment with drugs that may have the potential to reduce mutation burden, such as interferon and ruxolitinib. We surmise that our findings deserve confirmation in other, larger cohorts of patients with PV treated with ruxolitinib.

Authors

Paola Guglielmelli,[1] Chiara Paoli,[1] Giulia Borgi,[1] Lorenzo Fagiolo,[1] Sara Bianchini,[1] Giuseppe Gaetano Loscocco,[1] Francesca Gesullo,[1] Fernanda di Vaio,[1] Cecilia Matteoli,[1] Fabiana Pancani,[1] Sofia Danesi,[1] Simona Pestelli,[1] Tiziano Barbui[2] and Alessandro M. Vannucchi[1]

[1]Center Research and Innovation of Myeloproliferative Neoplasms-

CRIMM, Department of Clinical and Experimental Medicine, University of Florence, and Hematology Unit, Azienda Ospedaliera Universitaria Careggi, Florence and [2]FROM Research Foundation, Azienda Socio-Sanitaria Territoriale Papa Giovanni XXIII, Bergamo, Italy

Correspondence:
A.M. VANNUCCHI - a.vannucchi@unifi.it

https://doi.org/10.3324/haematol.2025.300245

Received: November 18, 2025.
Accepted: April 1, 2026.
Early view: April 9, 2026.

©2026 Ferrata Storti Foundation
Published under a CC BY-NC license

Disclosures

PG has received honoraria for lectures and advisory board participation from Incyte, AOP, Novartis, GSK, Takeda and Thermo Fisher Scientific. AMV has received honoraria for lectures and advisory board participation from Incyte, AOP, Bluemedicine, Menarini Stemline, Novartis, and GSK. TB has received honoraria for lectures and advisory board participation from AOP and GSK. The other authors have no conflicts of interest to disclose.

Contributions

PG, CP, TB, and AMV designed research, analyzed data, and drafted and finalized the manuscript. GB, LF, and GGL provided patients' data. SB, FdV, CM, and SD contributed to data collection and analysis. FG, FP, and SP contributed to sample collection and molecular analysis.

Funding

This work was supported by Associazione Italiana per la Ricerca sul Cancro (AIRC) 5×1000 call "Metastatic disease: the key unmet need in oncology" to MYNERVA (MYeloid NEoplasms Research Venture AIRC), project #21267.

Data-sharing statement

Aggregate data are available upon reasonable request to the corresponding author.

References

1. Furman D, Campisi J, Verdin E, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019;25(12):1822-1832.
2. Carobbio A, Vannucchi AM, De Stefano V, et al. Neutrophil-to-lymphocyte ratio is a novel predictor of venous thrombosis in polycythemia vera. Blood Cancer J. 2022;12(2):28.
3. Barbui T, Carobbio A, Ghirardi A, et al. Neutrophil-to-lymphocyte ratio as a prognostic indicator of mortality in polycythemia vera: insights from a prospective cohort analysis. Blood Cancer J. 2024;14(1):195.
4. Barbui T, Carobbio A, Guglielmelli P, et al. Neutrophil/lymphocyte ratio identifies low-risk polycythaemia vera patients for early ropeginterferon alfa-2b therapy. Br J Haematol. 2024;205(6):2287-2294.
5. Larsen MK, Skov V, Kjaer L, et al. Neutrophil-to-lymphocyte ratio and all-cause mortality with and without myeloproliferative neoplasms-a Danish longitudinal study. Blood Cancer J. 2024;14(1):28.
6. Barbui T, Ghirardi A, Empson V, et al. Neutrophil-to-lymphocyte ratio as surrogate for JAK2V617F suppression and event-free survival in polycythemia vera. Blood Cancer J. 2025;15(1):132.
7. Song J, Kim SJ, Gollamudi J, Thiagarajan P, Prchal JT. Downregulated KLF2 in polycythemia vera and essential thrombocythemia induces prothrombotic gene expression. Blood Adv. 2023;7(5):712-717.
8. Reeves BN, Kim SJ, Song J, et al. Tissue factor activity is increased in neutrophils from JAK2 V617F-mutated essential thrombocythemia and polycythemia vera patients. Am J Hematol. 2022;97(2):E37-E40.
9. Choi DC, Messali N, Uda NR, et al. JAK2V617F impairs lymphoid differentiation in myeloproliferative neoplasms. Leukemia. 2024;38(11):2487-2491.
10. Kiladjian JJ, Klade C, Georgiev P, et al. Event-free survival in early polycythemia vera patients correlates with molecular response to ropeginterferon alfa-2b or hydroxyurea/best available therapy (PROUD-PV/CONTINUATION-PV). Hemasphere. 2025;9(5):e70137.
11. Gorantla SP, Rassner M, Crossley KA, et al. Efficacy of JAK1/2 inhibition in murine myeloproliferative neoplasms is not mediated by targeting oncogenic signaling. Nat Commun. 2025;16(1):4833.
12. Vannucchi AM, Kiladjian JJ, Griesshammer M, et al. Ruxolitinib versus standard therapy for the treatment of polycythemia vera. N Engl J Med. 2015;372(5):426-435.
13. Passamonti F, Griesshammer M, Palandri F, et al. Ruxolitinib for the treatment of inadequately controlled polycythaemia vera without splenomegaly (RESPONSE-2): a randomised, open-label, phase 3b study. Lancet Oncol. 2017;18(1):88-99.
14. Harrison CN, Nangalia J, Boucher R, et al. Ruxolitinib versus best available therapy for polycythemia vera intolerant or resistant to hydroxycarbamide in a randomized trial. J Clin Oncol. 2023;41(19):3534-3544.
15. Guglielmelli P, Mora B, Gesullo F, et al. Clinical impact of mutated JAK2 allele burden reduction in polycythemia vera and essential thrombocythemia. Am J Hematol. 2024;99(8):1550-1559.