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Serum erythropoietin levels in 696 patients investigated for erythrocytosis with JAK2 mutation analysis

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CONFLICT OF INTEREST

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Serum erythropoietin levels in 696 patients investigated for erythrocytosis with *JAK2* mutation analysis

To the Editor:

Erythrocytosis is a common reason for referral to hematology, primarily to exclude polycythemia vera (PV), which has a high morbidity and mortality if untreated.[1] Recent changes to the World Health Organization (WHO) definition of PV reduced the hemoglobin thresholds required for diagnosis, leading to overlap with the normal range and resulting in more frequent testing.[2] In addition to a focused history for possible secondary causes, distinguishing PV from secondary erythrocytosis often requires laboratory investigation, including serum erythropoietin (EPO) measurement and/or molecular testing for *JAK2* mutations. Although *JAK2* mutations are found in other myeloproliferative neoplasms, in patients with erythrocytosis, presence of *JAK2* mutations is highly sensitive and specific for PV.[3] Nonetheless, molecular testing can be costly and relies on access to a specialized laboratory.

Various approaches to the investigation of erythrocytosis are found in the literature: some start with EPO measurement,[4] while others advocate concurrent EPO and *JAK2* testing.[5] In a recent issue of this journal, Tefferi and Barbui[3] recommend upfront *JAK2* mutation screening in patients with suspected PV. The sequential approach starting with EPO measurement is premised on a normal or high EPO level having a high negative predictive value (NPV) to rule out PV. In contrast, the justification for concurrent or upfront *JAK2* testing is one of clinical expediency in patients with a high pretest probability for PV.

Most evidence for EPO's utility in the investigation of erythrocytosis predates the advent of molecular testing for PV,[6] and the added value of EPO measurement beyond *JAK2* testing has been brought into question by more recent studies.[7] This descriptive study

revisited the utility of EPO measurement in the era of *JAK2* testing by examining serum EPO distribution in a large real-world cohort of patients with erythrocytosis referred to our center, an academic healthcare organization, which serves a population of approximately 2 million in Ontario, Canada.

We reviewed all patients aged 18 years or older investigated for erythrocytosis (>160 g/L for women or >165 g/L for men) with *JAK2* mutation testing between January 1, 2015, and May 12, 2021, extracting data on *JAK2* mutation analysis, serum EPO levels, final diagnosis, and risk factors for secondary erythrocytosis. All patients with available EPO levels were included. *JAK2* testing was performed by either quantitative polymerase chain reaction (qPCR) using the Roche 480 LightCycler (La Roche AG, Switzerland), single nucleotide polymorphism (SNP) allelotyping using the Agena MassARRAY system (Agena Biosciences, USA), or next generation sequencing (NGS) panel using the Oncomine Myeloid Research Assay (ThermoFisher Scientific, USA). qPCR and SNP allelotyping assays tested for *JAK2V617F* mutations; the NGS panel screened for any *JAK2* mutations in exons 12–15, in addition to sequence variants in 40 other genes (including *SH2B3*) and 29 fusion driver genes associated with myeloid malignancies. Serum EPO levels were measured by chemiluminescent immunoassay (Unicel DXi 800; Beckman Coulter, USA) with a normal range of 2.6–18.5 mIU/mL. We performed receiver operating characteristic (ROC) analysis to evaluate the diagnostic accuracies of different EPO levels in predicting *JAK2*-positive PV. Given the high morbidity and mortality of untreated PV, we used a high NPV (>99%) to determine the threshold EPO level needed to exclude a diagnosis of PV.

Over the 5-year study period, a total of 883 patients referred for erythrocytosis underwent molecular testing. Of these patients, 696 (78.8%) had EPO levels measured; most were ordered simultaneously with *JAK2* testing. Patient characteristics are shown in Table S1, stratified by *JAK2* mutation status. The final diagnosis was PV in all 72 patients with *JAK2* mutations (10.3%), and the remaining 624 patients (89.7%) were diagnosed with secondary causes of erythrocytosis, most commonly smoking (42.5%), obstructive sleep apnea (32.7%), chronic obstructive pulmonary disease (14.6%), and testosterone use (10.3%). Distribution of risk factors for secondary erythrocytosis is shown in Table S2. The median EPO levels for *JAK2*-positive and *JAK2*-negative patients were 1.8 mIU/mL (range 0–26.4 mIU/mL) and 9.2 mIU/mL (range 1.3–762 mIU/mL), respectively ($p < .001$). There was a considerable overlap in EPO levels between *JAK2*-positive and -negative patients, with 80.2% ($n = 559$) of our cohort falling within the normal range (Figure 1A). Of the *JAK2*-positive patients, 25 (34.7%) had EPO levels at or above the lower limit of normal (≥2.6 mIU/mL). One *JAK2*-positive patient had an EPO level above 18.5 mIU/mL, which may have been explained by concomitant heavy smoking. EPO testing was performed at the time of initial consultation in all patients; no patients were identified as being on active treatment for erythrocytosis, either in the form of phlebotomy or cytoreductive therapy, at the time of measurement. Of the *JAK2*-positive patients, 34.7% ($n = 25$) had a prior history of thrombosis at the time of initial presentation, with the majority (84%) being arterial events. Of the *JAK2*-negative patients with subnormal EPO levels ($n = 15$), potential confounding factors included acute kidney injury ($n = 4$), chronic kidney disease ($n = 2$), and liver disease ($n = 5$); none was identified as having EPO receptor mutations.

ROC analysis established 4.75 mIU/mL as the optimal cut point; EPO levels below this threshold had a sensitivity of 90.3% and specificity of 88.0% for predicting a diagnosis of PV (area under the curve [AUC] = 0.94, Figure 1B). For use as an initial screening test to exclude *JAK2*-positive PV, a higher sensitivity (95.8%) could be achieved by increasing the EPO threshold to 7.15 mIU/mL. An EPO level > 27 mIU/mL was required to achieve a sensitivity of 100% and a NPV of 100% to effectively rule out *JAK2*-positive PV. Applied to our cohort, only 4.3% ($n = 30$) were at or above this threshold, meaning that an EPO level could have been used to rule out *JAK2*-positive PV.

Given the significant number of PV patients with normal EPO levels (≥2.6 mIU/mL), we reviewed secondary causes that might potentially explain "inappropriately normal" EPO levels in these patients. We found that risk factors for secondary erythrocytosis were overrepresented in PV patients with normal/high vs. low EPO levels, including smoking (40.0% vs. 14.9%) and obstructive sleep apnea (16.0% vs. 4.3%), but only smoking reached statistical significance ($p = .022$).

In summary, these data on serum EPO distribution in a large real-world cohort of patients referred for suspected PV suggest that EPO measurement may have limited added value when used concurrently with *JAK2* testing to rule out PV. We demonstrated that normal EPO levels are present in over a third of patients with *JAK2*-positive PV and therefore do not distinguish between PV and secondary erythrocytosis. A higher EPO threshold (>7.15 mIU/mL) showed improved sensitivity (95.8%), suggesting that EPO testing may still play an initial role if *JAK2* testing is not available. Nonetheless, in our cohort, a very high EPO threshold (>27 mIU/mL) was required to rule out PV with sufficient confidence to avoid *JAK2* testing, a level that would have excluded PV in only a small proportion of patients (4.3%).

These data question the value of simultaneous EPO and *JAK2* testing in the diagnosis of PV. EPO may retain diagnostic utility in sequential testing; however, our findings challenge the premise implicit in many sequential algorithms that normal or high EPO levels are sufficient to exclude PV. Implementation of a diagnostic algorithm and selection of EPO thresholds must be based on local disease prevalence and test performance rather than normal reference ranges for EPO, which vary depending on population characteristics and laboratory methods, and are difficult to extrapolate between settings. The diagnostic utility of EPO is further confounded by preanalytical variables, which include diurnal variation, as well as risk factors for hypoxia such as smoking, obstructive sleep apnea, and place of residence. In our cohort, smoking was overrepresented in PV patients with normal or high EPO levels, in keeping with previous findings.[7]

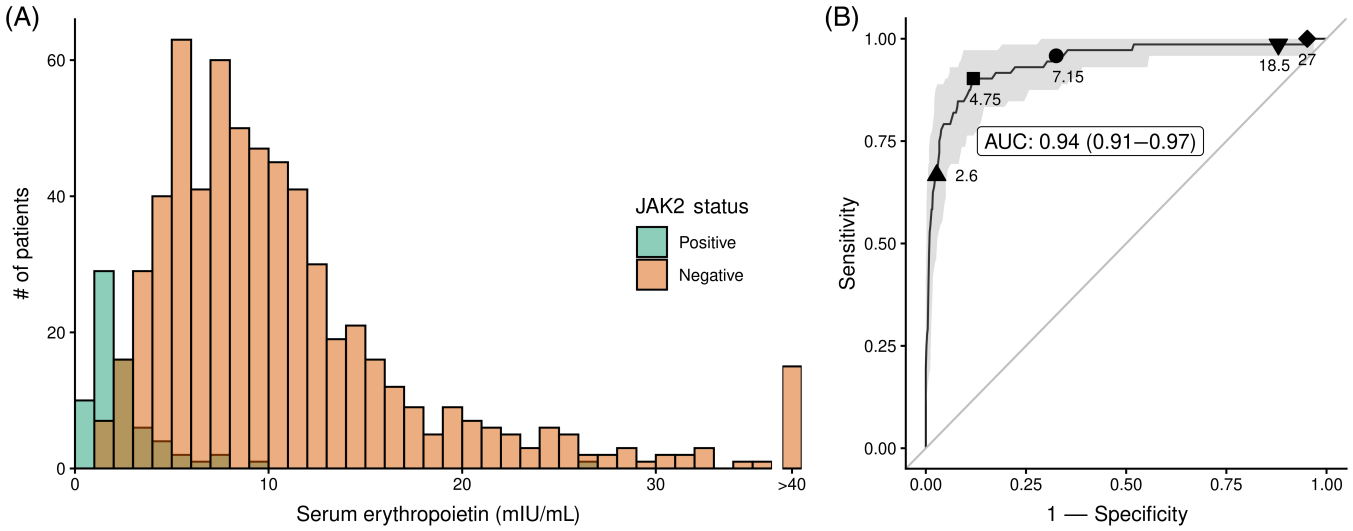

FIGURE 1 (A) Distribution of erythropoietin (EPO) levels *JAK2*-positive ($n = 72$) and negative patients ($n = 624$); (B) Receiver operating characteristic (ROC) curve for EPO as a predictor of *JAK2*-positive polycythemia vera (PV). Shaded area indicates 95% confidence intervals. Points indicate different EPO thresholds: ▲ = 2.6 mIU/mL (lower limit of normal), sensitivity 67%, specificity 97%; ■ = 4.75 mIU/mL (optimal cut point), sensitivity 90%, specificity 88%; ● = 7.15 mIU/mL (threshold with sensitivity > 95%), sensitivity 96%, specificity 67%; ▼ = 18.5 mIU/mL (upper limit of normal), sensitivity 99%, specificity 12%; ◆ = 27 mIU/mL (threshold with NPV >99%), sensitivity 100%, specificity 5%

Limitations include our study's retrospective design, making us unable to control for variables such as variation in timing of EPO measurement and method of *JAK2* testing. Our study was conducted at a tertiary referral center with ready access to molecular diagnostics: it does not address the utility of EPO measurement in low resource settings or at centers with limited access to *JAK2* testing. In such settings, EPO testing may be helpful provided that the user is aware of the performance characteristics of the assay in their patient population. Although our cohort is more representative of all patients referred to specialty hematology clinics for erythrocytosis compared to previous studies examining the utility of EPO testing,[6] the prevalence of PV in our study remains higher than in population-based cohorts; in lower prevalence settings, initial EPO testing may have greater utility in excluding PV. Additionally, EPO measurement plays a diagnostic role in the rare subgroup of PV patients without *JAK2V617F* or exon 12 mutations.[3] Given our objective to address the practical utility of EPO measurement in patients referred for erythrocytosis, we used elevated hemoglobin levels as an inclusion criteria for our study; we acknowledge this is an imperfect surrogate for red cell mass, which is no longer routinely measured in practice.

Despite these caveats, our study supplements existing literature on the limited utility of EPO measurement with data from a large cohort of patients investigated for erythrocytosis with *JAK2* testing. Our findings are consistent with clinical experience that EPO levels often have limited value in discriminating between primary and secondary causes of erythrocytosis given that most results are in the normal range at values that can neither rule out nor rule in PV. Even for the limited number of patients with elevated EPO levels, these levels rarely reach a threshold sufficient to exclude a diagnosis of PV with a high NPV.

While EPO measurement is generally less costly than molecular diagnostics, the common practice of simultaneously ordering EPO levels and *JAK2* mutation analysis negates any potential cost savings. In patients with a high pretest probability of PV, however, the sequential approach is likely not a cost-effective strategy for investigating erythrocytosis and may only add to costs in the form of unnecessary testing, repeat clinic visits and diagnostic delays, justifying the approach of upfront *JAK2* mutation screening recently proposed by Tefferi and Barbui.[3] With declining costs of molecular diagnostics, an updated cost–benefit analysis is needed to better define the role of EPO testing in the work up of PV. Given rising referrals for suspected PV in the wake of the 2016 WHO revision, further research should also examine whether alternate clinical or laboratory data aside from EPO levels, such as lactate dehydrogenase levels or complete blood count parameters,[8] might be used to more effectively triage *JAK2* testing in patients with erythrocytosis.

AUTHOR CONTRIBUTIONS

This study was designed by Benjamin Chin-Yee, Ian Chin-Yee, and Cyrus C. Hsia. Molecular diagnostic data were provided by Bekim Sadikovic, Pratibha Bhai, and Hanxin Lin. Clinical and laboratory data were collected by Ian Cheong, Maxim Matyashin, Benjamin Chin-Yee, Cyrus C. Hsia, and Ian Chin-Yee. Data analysis was performed by Ian Cheong, Alejandro Lazo-Langner, and Vipin Bhayana. Benjamin Chin-Yee wrote the article with input from all authors who approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Real-world applicability of commercial chimeric antigen receptor T-cell therapy among older adults with relapsed and/or refractory multiple myeloma

To the Editor:

Despite significant advances in the treatment of multiple myeloma (MM), most patients inevitably relapse and ultimately succumb to their disease. In particular, the treatment of patients who are triple-class refractory (TCR, i.e., refractory to an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 antibody) remains a significant challenge with a median overall survival of 9 months and limited therapeutic options available.[1,2] In 2021, the US Food and Drug Administration approved Idecabtagene vicleucel, the first chimeric antigen receptor-T-cell (CAR-T) therapy, for patients with relapsed/refractory multiple myeloma (RRMM) who have received at least four prior lines of therapy. Approval was based on the single arm phase II KARMMA study among 128 patients with heavily pretreated MM (84% TCR) demonstrating an overall response rate of 73%, complete response rate of 33%, and a median progression-free survival of 8.8 months; efficacy parameters considered transformative in this setting.[3]

While the FDA approval of CAR-T therapy among patients with RRMM represents a significant addition to the therapeutic arsenal, its immediate benefit in real-world patients with MM, particularly older adults, is unknown. Published real-world evidence suggests that 32–61% and 14–38% of patients with symptomatic MM receive second- and third-line therapy, respectively.[4] Furthermore, the very reeligibility criteria used by the phase II KarMMa study may further limit the number of patients who are considered for this therapy.

We used the Flatiron Health Electronic Health Record (EHR)-derived deidentified database, a longitudinal database comprising patient-level structured and unstructured data, curated via technology-enabled abstraction, originating from approximately 280 cancer clinics across the United States (≈800 sites of care). Our