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Title: Sodium-glucose cotransporter-2 inhibitor use is common and underrecognized in patients investigated for erythrocytosis: a retrospective cohort study
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Reviewer 1: Matthew Kang
Institution: Joseph Brant Memorial Hospital
General comments (author response in bold)

This is an excellent retrospective study, with a large sample size, that highlights an important and overlooked factor when assessing patients with erythrocytosis.
We thank the reviewer for the positive comments and summary.

Reviewer 2: Dr. Siraj Mithoowani
Institution: McMaster University
General comments (author response in bold)

Drs. Chin-Yee et al conducted a retrospective cohort study on the use of JAK2 molecular testing as part of the diagnostic workup for patients with erythrocytosis. They show that JAK2 testing is being ordered with increasing frequency which mirrors our own local experience. They also demonstrate a convincing association between SGLT2 inhibitor use and erythrocytosis. I would like to congratulate the authors on this important work. Their paper is timely and provides practical recommendations on reducing unnecessary healthcare expenditures.
We thank the reviewer for the positive comments and summary.

Major concerns:
1) The authors' conclusion that the main driver for increased testing is "related to increased awareness of and accessibility to molecular diagnostics rather than a change in diagnostic criteria for PV" is overstated. One limitation is that the data only go back as far as Aug 2015 so most of the tests were ordered after publication of the WHO 2016 revision. Another is that referral volumes weren't captured - perhaps community physicians were simply more likely to refer patients with mild erythrocytosis (< 165 g/L in women or < 185 g/L in men) after the WHO criteria were revised, leading to a greater number of patients seen (and JAK2 tests performed) in specialty clinics. I suspect this is probably true given that EPO levels were also ordered more frequently over time (p.6) which suggests a greater awareness of PV, in general, during the study period. To further explore the impact of the WHO diagnostic criteria revision, it might be more helpful to report the proportion of JAK2 tests done in patients with different severities of erythrocytosis over time.
Thanks for making this point. Yes, we agree that based on these data it is not possible to infer the exact cause of the increases in molecular testing over time. We acknowledge that the time period examined may not give a clear picture of the trend overtime; however, this was necessary based on data available from our databases as mentioned in our responses above.
The suggestion to examine the rates of JAK2 testing in patients stratified by erythrocytosis severity is an interesting one; however, this would require a separate, population-based study and is beyond the scope of our present study.

In response to this comment we have modified the sentence in question to avoid overstating the drivers of increased testing, and have also acknowledged the reviewer's point in our limitations section. Thank you for these helpful insights.
"Measuring changes in referral volumes, as well as rates of JAK2 testing stratified by erythrocytosis severity may have provided a better means of ascertaining the impact of the change in WHO diagnostic criteria on the investigation of suspected PV." (p. 7)

2) I would appreciate if the authors' could comment on the proportion of tests done by the more expensive NGS panel (and the diagnostic yield) compared to the cheaper JAK2 V617F targeted assay in their cohort. Non-V617F mutations are extremely rare, so perhaps limiting the number of NGS tests would be an easier way to reduce healthcare costs compared to recommending that EPO and JAK2 V617F tests are ordered sequentially (rather than concurrently -- which I still think is reasonable in settings where the pre-test probability of PV is high).
Thanks for this question. We share the reviewer's opinion that the role of upfront NGS testing (which tests for V617F and exon 12 mutations) vs. targeted JAK2V617F testing remains to be defined in the investigation of erythrocytosis, and we agree that with this comment that limiting the number of NGS tests may be another, potentially easier, way to reduce costs. Answering such a question would require a cost-benefit analysis that is beyond the scope of the current study, but we are currently undertaking a quality improvement initiative at our institution to better define the diagnostic pathway in the evaluation of erythrocytosis that will provide the optimal approach, considering both diagnostic sensitivities and cost. For the purposes of this study, both assays offered high sensitivities to effectively rule out polycythemia vera in patients who underwent molecular testing. To answer the reviewer's question, the majority of patients (n = 529) in our study had testing by NGS rather than the targeted assay, which reflects funding for this testing that was available at our institution during the study period to cover the cost of NGS testing. The cost implications of this approach was not assessed, but likely suggests further opportunities for improvement in resource utilization in the investigation of erythrocytosis, in line with the main findings of our article.
In response to the reviewer's comment, we have added mention of this consideration of the cost of NGS versus targeted our discussion section:
"Our study included patients who had JAK2 mutation testing by either NGS panel or targeted assays; the role of each method in the diagnostic work up of erythrocytosis remains to be defined but given the higher cost of NGS, prioritizing targeted assays may offer another means of reducing costs in the investigation of patients with suspected PV." (p. 7)

3) The authors state several hypotheses why SGLT2 inhibitors might lead to erythrocytosis. What about the mild diuretic effect of these drugs? Perhaps an analogous situation would be to look at diuretic use in your cohort, which is also an important (and potentially under-recognized) cause of relative erythrocytosis.
This is a great point, as there may be other medications or clinical scenarios that can lead to erythrocytosis such as novel therapies or procedures, vaping, and other factors that we may not know yet if there is an association. However, in the case of diuretics, that effect is well known and well described as a transient cause of relative (or apparent) erythrocytosis.

We collected data on all medications at the time of molecular testing, and the only medications that were found to be associated with erythrocytosis were testosterone and SGLT2-inhibitors; we did not find an association with diuretic use.
This may reflect the fact that, in our cohort, these were patients who were initially assessed by family physicians or internal medicine specialists already by the time they were referred to hematology for erythrocytosis, which may have selected outpatients with transient or relative causes of erythrocytosis.
In terms of pathophysiology or mechanism of action of SGLT2 inhibitor associated erythrocytosis, this was previously published in CMAJ https://www.cmaj.ca/content/192/42/E1271 and referenced in this study.
In response to the reviewer's comment we have added mention of the mild diuretic effect in the discussion on postulated mechanisms.
"The mechanisms of SGLT2 inhibitor-induced erythrocytosis remains to be elucidated but have been postulated to include hemoconcentration due to mild diuretic effect, modulation of iron metabolism by suppression of hepcidin (9), as well as stimulation of renal erythropoietin production (10)." (p. 6)

Minor concerns:
1) In addition to reporting the average hemoglobin values in your cohort (p. 4) I also suggest also reporting average hematocrit values. As you know, the hematocrit thresholds to diagnose PV were also adjusted downward in the WHO 2016 diagnostic criteria (>0.48 in women, 0.49 in men). In my anecdotal experience, high hematocrit with "normal" hemoglobin is a common reason for referral to the benign hematology clinic nowadays.
This is a great point that high hematocrit may be a trigger for referral to a specialist for work-up. It should be pointed out that in most labs including our own older analyzer the hematocrit is a calculated value.
Our Beckman coulter analyzer, hematocrit is based on MCV x RBC count. This was replaced a by Sysmex XN series analyzer. The haematocrit (HCT) is obtained from the cumulative values of the individual cell pulse heights from a direct measurement and then added up. In our data, erythrocytosis was defined by elevated haemoglobin and in this cohort the hematocrit ranged from 0.46-0.73 in men and 0.45-0.73 in women.
As per our response above, we have added a Table 1 with patient characteristics, which includes hematocrit values.