Sodium-glucose cotransporter-2 inhibitor use is common and underrecognized in patients investigated for erythrocytosis: a five-year retrospective cohort study

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Abstract

Background: Molecular testing for *JAK2* mutations is part of the standard diagnostic workup for patients with suspected polycythemia vera; however, secondary causes of erythrocytosis remain the most common in practice. This study characterizes evolving practice patterns in the investigation of erythrocytosis and the prevalence of secondary causes among patients who underwent molecular testing.

Methods: We reviewed all consecutive patients investigated for erythrocytosis (>160 g/L for women; >165 g/L for men) with *JAK2* testing between 2015 and 2021 at a tertiary referral centre in Ontario, Canada to assess changes in *JAK2* mutation positivity rates, average hemoglobin levels, and prevalence of secondary causes of erythrocytosis.

Results: A total of 891 patients with erythrocytosis underwent *JAK2* mutation testing with an increase in number of tests, decrease in *JAK2* positivity rates, and similar average hemoglobin levels over the five-year study period. We observed a high prevalence of secondary causes of erythrocytosis among patients who underwent molecular testing (59-74%) and an increase in use of medications associated with erythrocytosis, including testosterone (6-11%) and sodium-glucose cotransporter-2 (SGLT2) inhibitors (2-19%). Discontinuation of SGLT2 inhibitors was associated with a significant decrease in hemoglobin levels (mean -14.7 g/L, 95% CI -18.9 to -10.5 g/L) compared to continuation ($P < 0.001$).

Interpretation: SGLT2 inhibitors may be a common and underrecognized cause of elevated hemoglobin levels in patients investigated for erythrocytosis. This effect of SGLT2 inhibitors has been observed in clinical trials and case reports but has not been previously reported at this scale. Our findings underscore the importance of a detailed medical history to support more judicious use of molecular testing in adherence with current guidelines on the investigation of erythrocytosis.

Introduction

Since the identification of the *JAK2 V617F* mutation in polycythemia vera (PV) in 2005 (1), molecular testing of *JAK2* has become part of the standard diagnostic workup for erythrocytosis. The increasing availability of molecular diagnostics and a decrease in hemoglobin thresholds required to diagnose PV in the revised 2016 World Health Organization (WHO) classification (2) are two potential factors driving increased utilization of molecular testing in patients with erythrocytosis.

Secondary causes of erythrocytosis, such as smoking, hypoxic lung diseases, and medications are more common than PV, and guidelines recommend first excluding these etiologies through history and focused investigations, including serum erythropoietin levels, before performing molecular testing (3). Despite these recommendations, in practice, this work up is often performed concurrently with molecular testing, which carries significant healthcare costs.

The primary objective of this study was to characterize evolving practice patterns in the investigation of erythrocytosis, focusing on the utilization of molecular testing for *JAK2* mutations in a real-world cohort of patients referred for elevated hemoglobin levels. We hypothesized that an increase in molecular testing from 2015 to 2021 might have resulted from decreased hemoglobin thresholds in the revised 2016 WHO diagnostic criteria for PV and/or from improved access to *JAK2* testing. A secondary objective was to assess the prevalence of secondary causes of erythrocytosis in patients who underwent *JAK2* testing, including use of testosterone and sodium-glucose cotransporter-2 (SGLT2) inhibitors, both established but potentially underrecognized secondary causes of erythrocytosis (3–5). These findings could inform approaches to the investigation of erythrocytosis and interventions to improve resource stewardship in molecular testing.

Methods

This study was conducted at London Health Sciences Centre, a tertiary referral center that serves a population of approximately 2 million in Southwestern Ontario, Canada. We retrospectively reviewed all consecutive patients aged 18 or older investigated at our center for erythrocytosis (>160 g/L for women; >165 g/L for men) with *JAK2* mutation testing between August 1, 2015 and May 20, 2021. *JAK2* mutation testing was performed by quantitative polymerase chain reaction

(qPCR) using the Roche 480 LightCycler (La Roche AG, Switzerland) between 2015 and 2017 and by single nucleotide polymorphism (SNP) allelotyping using the Agena MassARRAY system (Agena Biosciences, USA) or Next Generation Sequencing (NGS) using the Oncomine Myeloid Research Assay (ThermoFisher Scientific, MA, USA) between 2018 and 2020. qPCR and SNP allelotyping assays were used to detect the *JAK2 V617F* mutation; the NGS assay was used to detect any *JAK2* mutations, including *V617F* and mutations in exons 12-15. We performed a chart review to extract laboratory and clinical data, including information on medical comorbidities and medications, with a focus on known secondary causes of erythrocytosis. Statistical analysis was performed in R (version 4.1.0) using Fisher's exact test and the Mann-Whitney *U* test. This study was approved by Research Ethics Boards at Western University (118139) and the Lawson Research Institute (10750).

Results

A total of 891 consecutive patients with erythrocytosis underwent *JAK2* mutation testing at our institution from August 1, 2015 to May 20, 2021. There was an overall increase in testing during the five-year study period, with a decline in the positive detection rate: 8/29 (28%) in 2015, 15/94 (16%) in 2016, 15/100 (15%) in 2017, 29/216 (13%) in 2018, 19/195 (10%) in 2019, 14/180 (8%) in 2020, and 6/77 (8%) in 2021 (*Figure 1*). The average hemoglobin levels in patients with erythrocytosis who underwent testing remained similar across all years (average 167-173 g/L for women, 95% confidence interval [CI] range 165-178 g/L; 179-181 g/L for men, 95% CI range 174-184 g/L).

In our cohort, there was a consistently high proportion of patients with documented secondary causes of erythrocytosis who underwent molecular testing, ranging from 59% to 74% from 2015 to 2021. Secondary causes included smoking, chronic obstructive pulmonary disease, obstructive sleep apnea, other hypoxic lung disease, erythropoietin-secreting tumor, or medications (*Figure 2*). We observed an increase in SGLT2 inhibitor use among patients who underwent *JAK2* testing, with 0/29 (0%) in 2015, 2/94 (2%) in 2016, 13/100 (13%) in 2017, 17/216 (8%) in 2018, 18/195 (9%) in 2019, 32/180 (18%) in 2020, and 15/77 (19%) in 2021. The proportion of patients on testosterone was relatively constant at 2/29 (7%) in 2015, 10/94 (11%) in 2016, 7/100 (7%) in 2017, 22/216 (10%) in 2018, 15/195 (8%) in 2019, 14/180 (8%) in 2020, and 5/77 (6%) in 2021 (*Figure 3*).

To ascertain if this observation was specific to patients with erythrocytosis we also reviewed all patients who underwent molecular testing for isolated thrombocytosis (platelet count >450 × 10^9/L) from 2018 to 2021; 5/446 (1.1%) patients with isolated thrombocytosis were on SGLT2 inhibitors, compared to 82/668 (12.3 %) patients with erythrocytosis during the same time period (P < 0.001). Likewise, a significantly lower proportion of patients with thrombocytosis were on testosterone compared to patients with erythrocytosis (2/446 [0.4%] vs. 55/668 [8.2%], P < 0.001). Rates of SGLT2 inhibitor and testosterone use were significantly higher in *JAK2*-negative compared to *JAK2*-positive patients (P < 0.001 for SGLT2 inhibitors; P < 0.01 for testosterone).

Of note, two patients on SGLT2 inhibitors were found to have *JAK2 V617F* mutations and were diagnosed with PV. Although hemoglobin levels of these two patients on presentation (both 170 g/L) were similar to the average of this cohort, both patients also had thrombocytosis and one had leukocytosis as well as associated symptoms suggestive of a myeloproliferative neoplasm, including aquagenic pruritus and unexplained weight loss. To evaluate the impact of SGLT2 inhibitors on erythrocytosis we reviewed all remaining patients on SGLT2 inhibitors in the cohort (n = 96). Follow up data were available for 91 patients. Sixteen patients had confounding factors, such as phlebotomy, acute illness, or other drug-induced cause (i.e., testosterone), precluding evaluation of follow up hemoglobin levels. Of the remaining 75 patients, 15 (20%) discontinued the SGLT2 inhibitor and 60 (80%) continued the medication. The average difference between the hemoglobin level at the time of referral and the maximum hemoglobin level during follow up was -14.7 g/L (95% CI -18.9 to -10.5 g/L) for those who discontinued SGLT2 inhibitors vs. 0.22 g/L (95% CI -1.77 to 2.33 g/L) for those who continued these medications (P < 0.001).

Discussion

This study describes changing patterns in the investigation of erythrocytosis in a real-world population, demonstrating an increase in molecular testing over time, with a decline in *JAK2* mutation detection rate. Although we initially hypothesized that an increase in *JAK2* testing may have resulted from a change in the WHO diagnostic criteria in 2016, the average hemoglobin levels remained relatively constant year-over-year in our study, suggesting that the main drivers of increased testing were likely related to increased awareness of and accessibility to molecular diagnostics rather than the change in diagnostic criteria for PV. This is further supported by the

high proportion of patients with documented secondary causes of erythrocytosis who underwent molecular testing over the period studied. Of note, we also observed an increase in serum erythropoietin testing over the same time period (data not shown), suggesting that a majority of clinicians ordered *JAK2* and erythropoietin tests concurrently, as suggested by some diagnostic algorithms (6).

One novel observation of this study was the increasing use of SGLT2 inhibitors among patients with erythrocytosis who underwent *JAK2* mutation testing. SGLT2 inhibitors are known to cause an increase in hemoglobin levels, an effect noted in initial clinical trials and hypothesized to contribute to their cardioprotective effects (7, 8). The mechanisms of SGLT2 inhibitor-induced erythrocytosis remains to be elucidated but have been postulated to include modulation of iron metabolism by suppression of hepcidin (9), as well as stimulation of renal erythropoietin production (10). In a post hoc analysis of the EMPA-REG OUTCOME trial, a mean increase in hemoglobin of 8 g/L was observed in the empagliflozin arms (8). Literature on more severe cases of erythrocytosis associated with SGLT2 inhibitor use is limited to a small number of case reports (11–13); however, the findings of our study suggests that this may be a more widespread phenomenon and that SGLT2 inhibitors may be a significant, underrecognized cause of drug-induced erythrocytosis. Indeed, at our center we have noted an increase in referrals for patients with erythrocytosis on SGLT2 inhibitors (5). SGLT2 inhibitors are an increasingly prescribed class of medications based on evidence of improved cardiovascular and renal outcomes (14), and indications have expanded beyond type 2 diabetes to also include chronic kidney disease and heart failure (15). Considering this growing use, practitioners should be aware of the potential for SGLT2 inhibitors to cause elevations in hemoglobin level to help limit over-investigation of patients with drug-induced erythrocytosis.

Molecular testing has revolutionized the diagnosis of PV but is associated with significant costs ranging from approximately $350 for *JAK2 V617F* targeted assays (e.g., qPCR) to $1000 for screening assays by NGS panel. Our study suggests that increased access to molecular diagnostics has been accompanied by less discriminate utilization. The high proportion of patients tested for *JAK2* mutations with suspected secondary causes of erythrocytosis underscores the importance of a detailed medical history and a medication review to support more judicious use of molecular testing. Medications associated with erythrocytosis such as testosterone or SGLT2 inhibitors are common, found in around 20% of patients our cohort, and easily identified from the history. In

such cases, a diagnostic/therapeutic trial of holding these medications should be considered, with molecular testing reserved for patients who fail to respond. Data from our study suggest that discontinuation of SGLT2 inhibitors was associated with a reduction and often normalization of hemoglobin levels, consistent with previous reports (13). Decisions to hold or discontinue these drugs should be made in collaboration with a patient's primary care physician and/or endocrinologist.

There are several limitations to our study. Firstly, this study was conducted at a single tertiary care center in Southwestern Ontario, Canada; practice patterns and access to molecular testing vary across Canada and our findings therefore may not be generalizable. Secondly, our study only included patients with elevated hemoglobin levels who underwent *JAK2* mutation testing. We did not examine all patients referred for erythrocytosis and thus were unable to measure a change in volume of referrals or the proportion of referred patients who went on to have molecular testing. Lastly, while we inferred less discriminate utilization of molecular testing from the high proportion of patients tested with known secondary causes of erythrocytosis, the presence of secondary causes does not exclude a diagnosis of PV, as observed in several patients in our cohort. These findings reinforce the need for an individualized approach to the investigation of erythrocytosis, guided by a focused history, including medication review, and adapted based on the results of ancillary testing and medication adjustments.

In conclusion, our study demonstrates an increase in *JAK2* mutation testing in patients with elevated hemoglobin levels over the past five years, most likely driven by increasing access to molecular diagnostics. This increase in molecular testing includes a large proportion of patients with known secondary causes of erythrocytosis, including a significant number on SGLT2 inhibitors. Our study can help increase awareness among providers of established and emerging secondary causes of erythrocytosis commonly encountered in practice and inform the development of more rational approaches to molecular testing, thus improving resource stewardship and lowering healthcare costs.

References

1. Kralovics R, Passamonti F, Buser AS et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352:1779-1790.
2. Arber DA, Orazi A, Hasserjian R et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391-2405.
3. Mithoowani S, Laureano M, Crowther MA, Hillis CM. Investigation and management of erythrocytosis. CMAJ. 2020;192:E913-E918.
4. Cervi A, Balitsky AK. Testosterone use causing erythrocytosis. CMAJ. 2017;189:E1286-E1288.
5. Chin-Yee B, Solh Z, Hsia C. Erythrocytosis induced by sodium-glucose cotransporter-2 inhibitors. CMAJ 2020;192(42):E1271.
6. Busque L, Porwit A, Day R et al. Laboratory Investigation of Myeloproliferative Neoplasms (MPNs): Recommendations of the Canadian MPN Group. Am J Clin Pathol. 2016;146:408-422.
7. Zinman B, Wanner C, Lachin JM et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med. 2015;373:2117-2128.
8. Inzucchi SE, Zinman B, Fitchett D et al. How Does Empagliflozin Reduce Cardiovascular Mortality? Insights From a Mediation Analysis of the EMPA-REG OUTCOME Trial. Diabetes Care. 2018;41:356-363.
9. Ghanim H, Abuaysheh S, Hejna J et al. Dapagliflozin Suppresses Hepcidin And Increases Erythropoiesis. J Clin Endocrinol Metab. 2020;E1056-E1063.
10. Mazer CD, Hare GMT, Connelly PW et al. Effect of Empagliflozin on Erythropoietin Levels, Iron Stores, and Red Blood Cell Morphology in Patients With Type 2 Diabetes Mellitus and Coronary Artery Disease. Circulation 2020;141(8):704-707.
11. Das L, Bhansali A, Walia R. Unmasking and aggravation of polycythemia vera by canagliflozin. Diabet Med. 2018;35:1613-1616.
12. Motta G, Zavattaro M, Romeo F, Lanfranco F, Broglio F. Risk of Erythrocytosis During Concomitant Testosterone and SGLT2-Inhibitor Treatment: A Warning From Two Clinical Cases. J Clinical Endocrinol Metab. 2019;104:819-822.
13. Gupta R, Gupta A, Shrikhande M, Tyagi K, Ghosh A, Misra A. Marked erythrocytosis during treatment with sodium glucose cotransporter-2 inhibitors-report of two cases. Diabetes Res Clin Pract. 2020;162:108127.
14. Brown E, Heerspink HJL, Cuthbertson DJ, Wilding JPH. SGLT2 inhibitors and GLP-1 receptor agonists: established and emerging indications. Lancet. 2021
15. O'Meara E, McDonald M, Chan M et al. CCS/CHFS Heart Failure Guidelines: Clinical Trial Update on Functional Mitral Regurgitation, SGLT2 Inhibitors, ARNI in HFpEF, and Tafamidis in Amyloidosis. Can J Cardiol. 2020;36:159-169.

Figures

Figure 1. Number of patients and *JAK2* positivity rates between 2015 and 2021. Lines indicate average hemoglobin levels for male and female patients tested each year with 95% CI. *2015 includes only patients tested from August 1 to December 31, 2015; ^2021 includes only patients tested from January 1 to May 20, 2021.

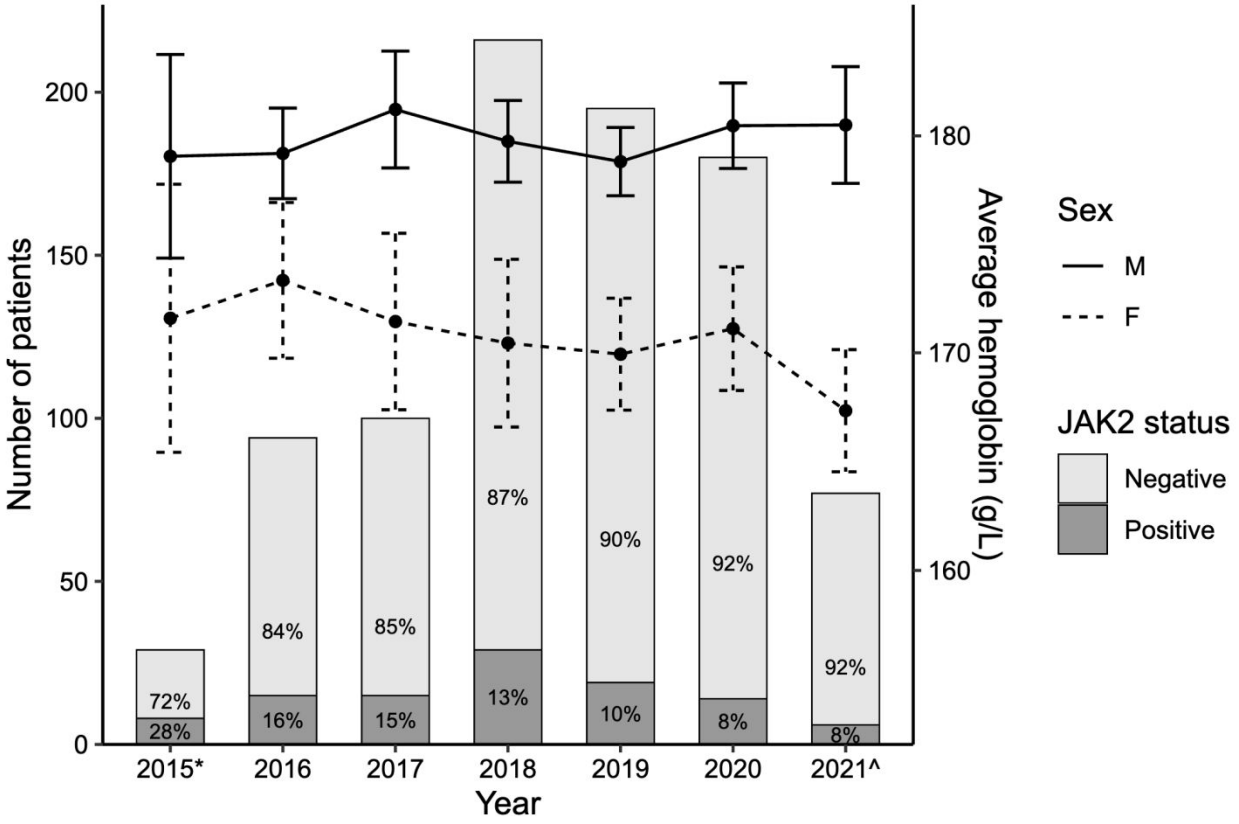


Figure 2. Distribution of secondary causes of erythrocytosis among *JAK2*-positive and -negative patients in our cohort. Differences in secondary causes between *JAK2*-positive and -negative patients were statistically significant for smoking ($P < 0.001$), OSA ($P < 0.001$), COPD ($P < 0.01$), SGLT2 inhibitors ($P < 0.001$), and testosterone use ($P < 0.01$). COPD, chronic obstructive pulmonary disease; OSA, obstructive sleep apnea; EPO, erythropoietin.

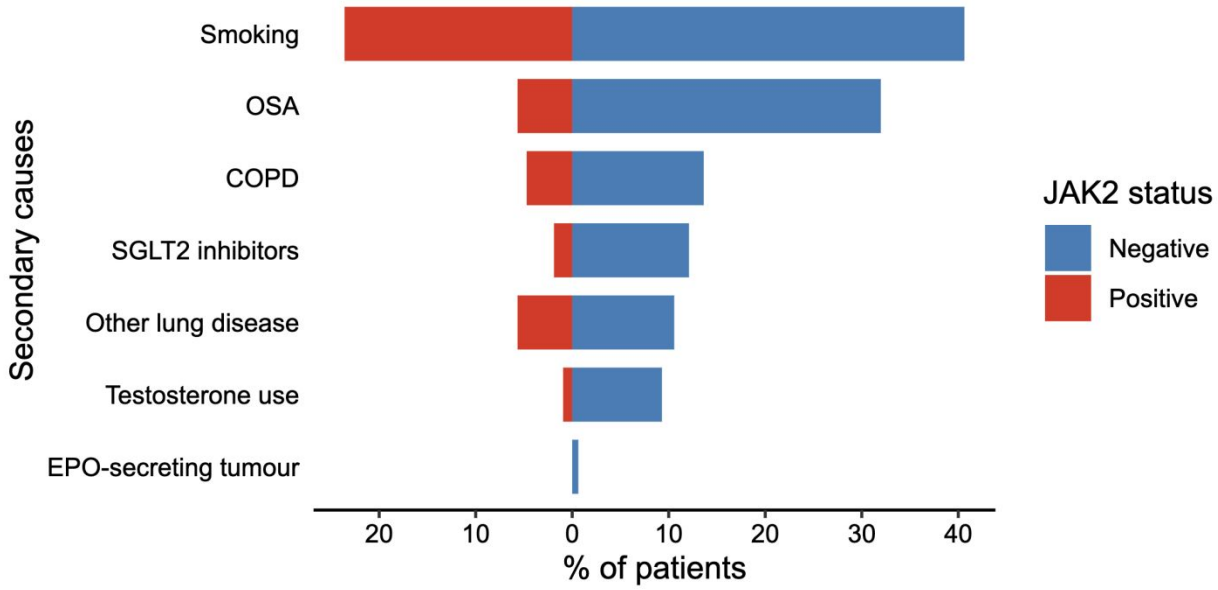
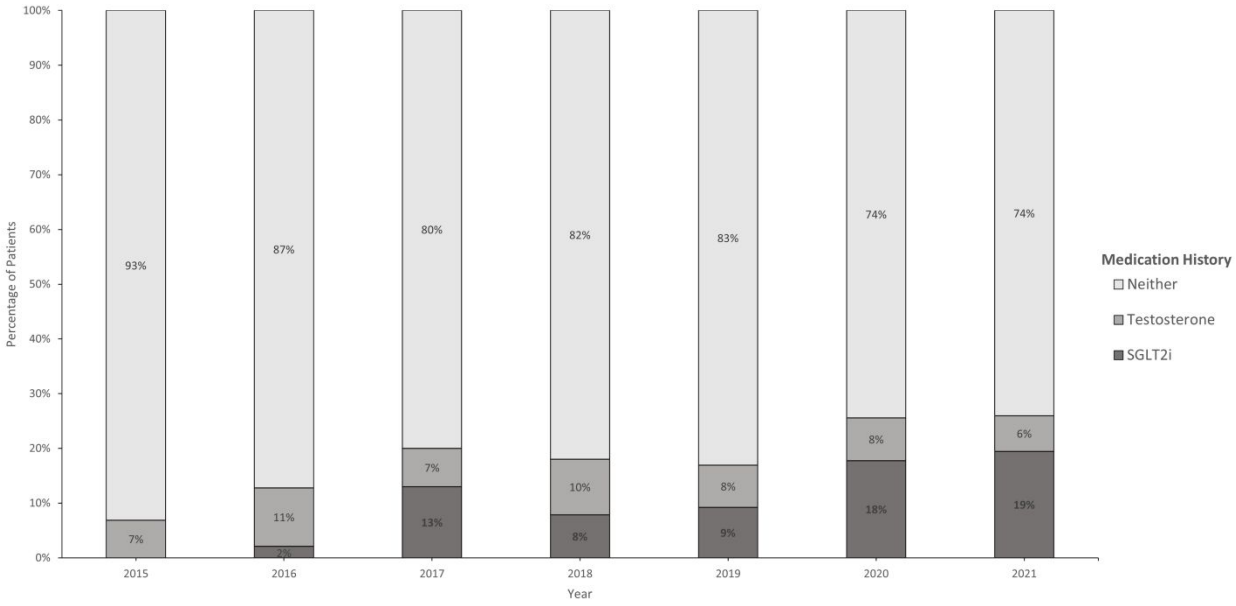
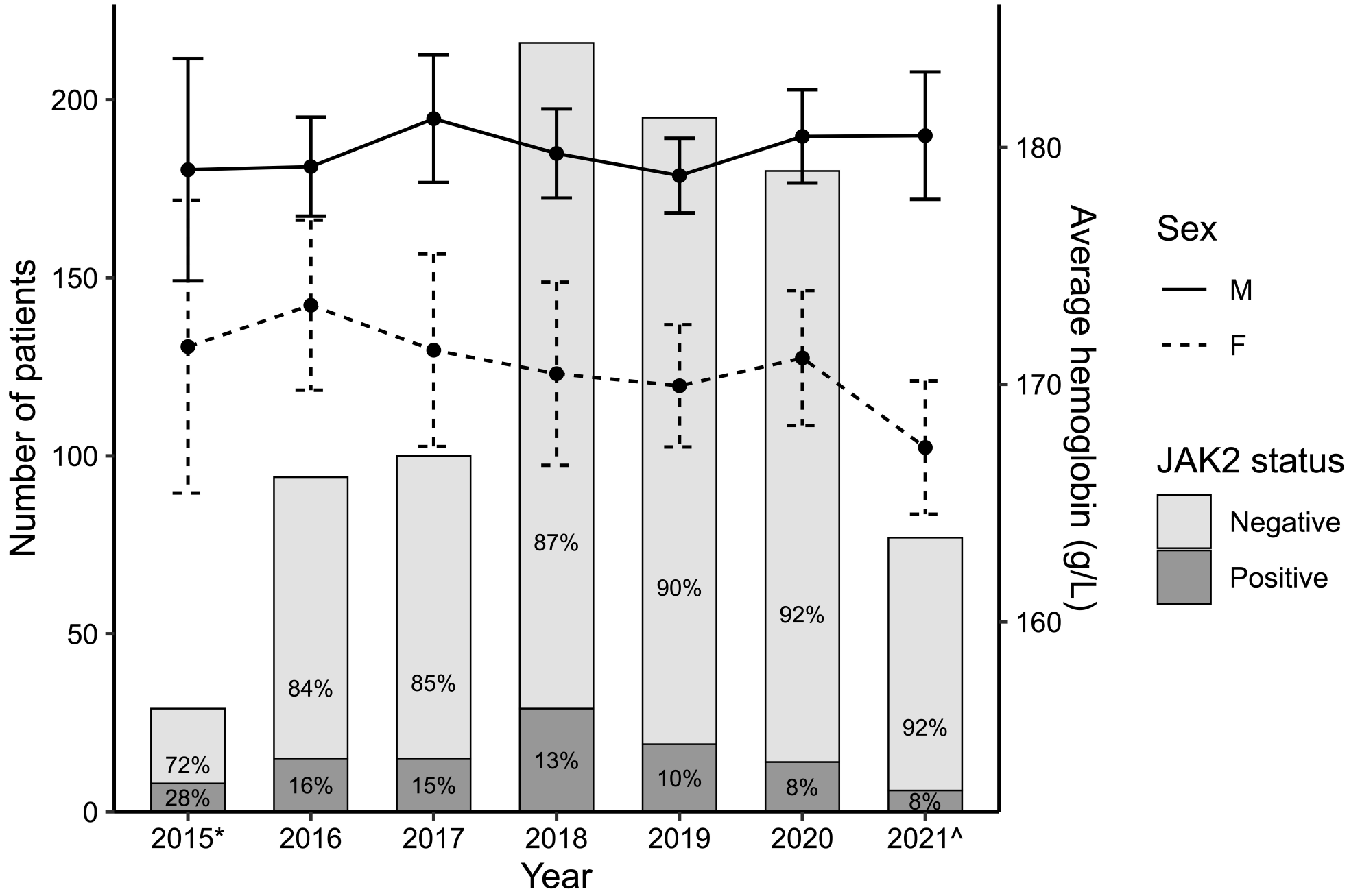
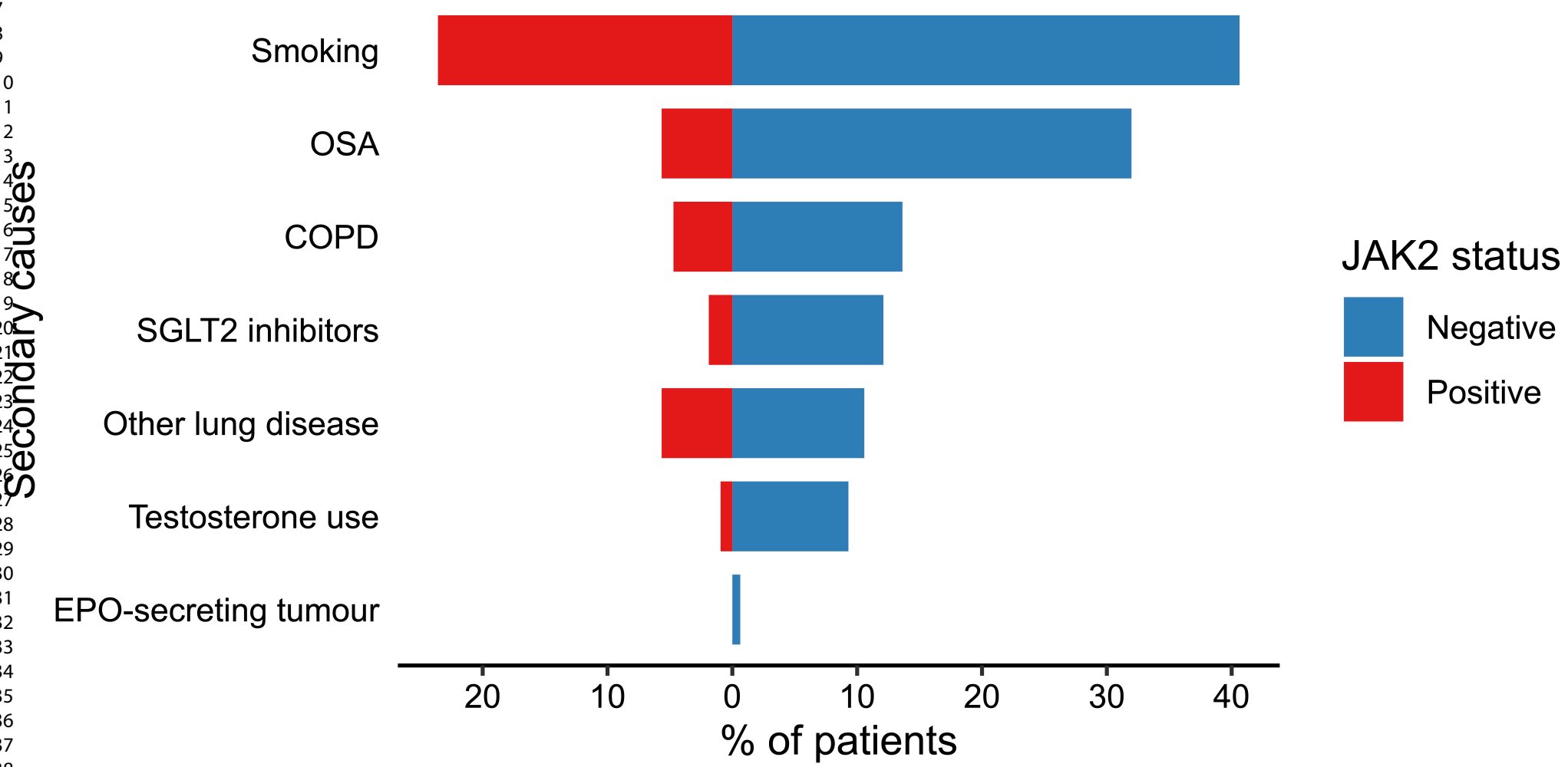
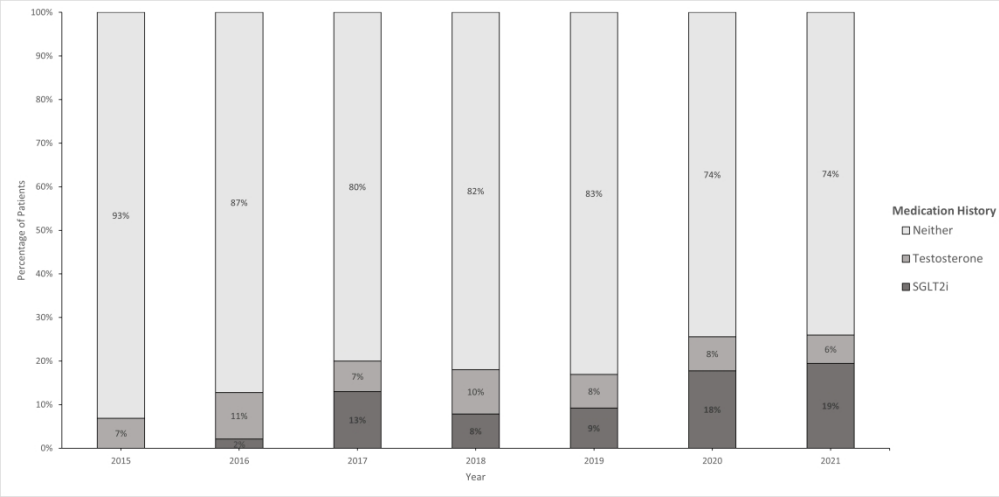


Figure 3. Use of testosterone and SGLT2 inhibitors among patients with erythrocytosis who underwent *JAK2* mutation testing between 2015 and 2021. Note, 4 patients in our cohort were on both testosterone and an SGLT2 inhibitor.









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