Hemochromatosis Gene Mutation in Persons Developing Erythrocytosis on Combined Testosterone and SGLT-2 Inhibitor Therapy

Kamilya A. Schumacher, BA[1] ⓘD
and Aidar R. Gosmanov, MD, PhD, FACE[1,2] ⓘD

Abstract

In clinical trials, sodium-glucose cotransporter-2 inhibitors (SGLT-2i) use alone in persons with type 2 diabetes (T2D) or testosterone replacement therapy (TRT) prescription alone in men with hypogonadism was shown to lead to a modest but significant increase in red blood cell mass. Recent evidence indicates that combined use of TRT and SGLT-2i in persons with T2D may be associated with risk of erythrocytosis. However, factor(s) that may lead to the development of erythrocytosis in these patients is unknown. We describe here 5 consecutive patients with hypogonadism on chronic TRT who developed erythrocytosis following addition of SGLT-2i empagliflozin for optimization of T2D management. In addition to the careful review of medical history, all patients underwent genetic screening for hereditary hemochromatosis. We have found that none of the patients had C282Y mutation in the HFE (Homeostatic Iron Regulator) gene and 4 out of 5 patients had heterozygosity in the H63D allele. Upon TRT discontinuation or its dose reduction or referral for scheduled phlebotomy, patients showed resolution of erythrocytosis. Our study reaffirms that practitioners should monitor for changes in hematocrit following the initiation of SGLT-2i in persons with T2D and hypogonadism on chronic TRT. Also, for the first time, we showed that in some of the patients receiving combined TRT and SGLT-2i H63D heterozygosity in the HFE gene may mediate the development of new-onset erythrocytosis.

Keywords

SGLT-2i, testosterone, erythrocytosis, hemochromatosis, HFE

Introduction

There is a growing body of evidence that sodium-glucose cotransporter-2 inhibitors (SGLT-2i) use in patients with type 2 diabetes (T2D) is associated with modest but significant increase in hematocrit (Hct). The data demonstrating this "off-target" phenomenon have been consistently reproduced in both registration trials and long-term clinical programs that studied currently approved SGLT-2i.[1-6] With expansion of clinical application of SGLT-2i use driven by their glycemic efficacy and cardiovascular and renal benefits, early real-world observations have signaled that the concomitant use of gliflozins and testosterone therapy in persons with T2D and hypogonadism may be associated with *de novo* development of erythrocytosis.[7] The recognition and understanding of risk factors leading to the erythrocytosis is important as the latter adverse finding may increase risk of thromboembolic and cardiovascular events.[8]

In clinical practice, new-onset erythrocytosis in adult patients is more likely to be associated with concomitant secondary conditions such as obstructive sleep apnea (OSA), tobacco use, and/or cardiac or other respiratory diseases known to lead to erythroid hyperplasia secondary to hypoxia.[8] Exogenous testosterone replacement therapy (TRT) in men with hypogonadism by itself can increase risk of erythrocytosis defined as Hct >54%; with this, there are clear

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[1]Albany Medical College, NY, USA
[2]Albany Stratton VA Medical Center, NY, USA

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Corresponding Author:
Aidar R. Gosmanov, MD, PhD, FACE, Section of Endocrinology, Albany Stratton VA Medical Center, 113 Holland Avenue, Room A738, Albany, NY 12208, USA.
Email: agosmanov@gmail.com

Table 1. Clinical and Biochemical Characteristics of the Study Patients.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age, y	71	69	63	63	56
Body weight, kg	102.3	93.2	117.2	107.3	106.6
BMI, kg/m²	29.3	30.1	39.2	34.9	31.1
Diabetes medications	Metformin, glipizide	Metformin, sitagliptin	Metformin, acarbose, glimepiride, alogliptin	Metformin, insulin U500, exenatide weekly	Glimepiride
TRT prescription	T. 1.62% gel, 4 pumps/d	T. cypionate 150 mg intramuscularly, every 10 days	T. 1.62% gel, 2 pumps/d	T. cypionate 150 mg intramuscularly, every 14 days	T. 1.62% gel, 4 pumps/d
Current tobacco use	No	No	No	No	No
Sleep apnea	No	No	No	Yes (treated)	No
Ejection fraction, %	45-50	n.d.	68	n.d.	49
Hemoglobin A$_{1c}$[a], %	8.6	7.4	8.7	8.8	7.0
eGFR, mL/min/1.73 m²	83	66	86	85	52
Hematocrit[a], %	43.4	49.1	46.0	45.0	45.7
Testosterone[b], ng/dL	184	155	216	156	280

Abbreviations: BMI, body mass index; TRT, testosterone replacement therapy; n.d., not done.
[a]Before initiation of empagliflozin.
[b]At the time of hypogonadism diagnosis.

recommendations allowing us to diminish such risk when TRT is prescribed.[9] To our knowledge, there are no follow-up publications confirming original observation of *de novo* erythrocytosis in men receiving TRT following SGLT-2i initiation[7] nor there are studies to describe if particular risk factors may explain such risk.

In our previous study, we reported a case of new diagnosis of hereditary hemochromatosis (HH) gene (Homeostatic Iron Regulator or HFE) mutation in a person who developed erythrocytosis following initiation of TRT.[10] HH is the most common autosomal recessive disorder in whites of European descent that results in uninhibited intestinal iron absorption, which may among other manifestations of the disease increase iron availability for erythroid cell precursors with subsequent risk of erythrocytosis.[11-13] Herein, we report 5 consecutive persons with T2D and hypogonadism on chronic TRT who developed new-onset erythrocytosis following initiation SGLT-2i therapy and describe for the first time the risk of HFE gene mutation in these patients.

Cases

Patient 1 was a 71-year-old white male (WM) with past medical history of T2D, coronary artery disease (CAD), hypertension, congestive heart failure (CHF), hypothyroidism, dyslipidemia, and hypogonadism who presented to endocrinology clinic in December 2017 for optimization of diabetes management (Table 1). At the time of evaluation, his hypogonadism was optimally managed by topical testosterone preparation which was started 15 months before the visit. To improve glycemic control, SGLT-2i empagliflozin 10 mg daily was initiated and within 6 months was uptitrated to 25 mg. Over 16 months following the initiation of empagliflozin therapy and while on stable TRT, his hemoglobin A$_{1c}$ has

improved to 6.3%; however, at the same time, we have noted gradual increase in Hct levels reaching highest value of 56.1% (normal 38%-50%) found during routine clinical visit which satisfied definition of erythrocytosis (Hct >54.0%).[9] We therefore discussed with the patient potential strategies to address new-onset erythrocytosis and he agreed to de-intensify TRT while continuing empagliflozin therapy. Gradual de-escalation of testosterone gel from 4 pumps to 2 pumps daily resulted in the normalization of Hct level (Figure 1). Considering unusual clinical scenario of the erythrocytosis development, we have conducted thorough evaluation of potential clinical or biochemical factors that could trigger this adverse event. He did not have history of OSA, current tobacco use, or underlying lung disease and his CHF was compensated. Hence, having identified no clear clinical triggers, we requested genetic analysis of common mutations in the HFE gene which revealed that the patient was negative for the C282Y pathogenic variant (C282Y –/–) and was heterozygous for the H63D mutation (H63D +/–). For last 2 years, while on most recent TRT and empagliflozin regimen, his Hct remains below 50%.

Patient 2 was a 69-year-old WM with past medical history of T2D, CAD, hypertension, dyslipidemia, and hypogonadism who presented to endocrinology clinic in March 2018 for optimization of diabetes management (Table 1). At that point, his hypogonadism was optimally managed by injectable testosterone preparation which was initially prescribed in 2011. To optimize glycemic control, empagliflozin 10 mg daily was initiated. Two months later, he presented to the emergency department complaining of progressive headaches. Extensive clinical, biochemical, and radiological evaluation has revealed at that time presence of hypertensive urgency, Hct of 56.5%, and hemoglobin A$_{1c}$ of 6.4%. Given acuity of his complaints and new diagnosis of erythrocytosis,
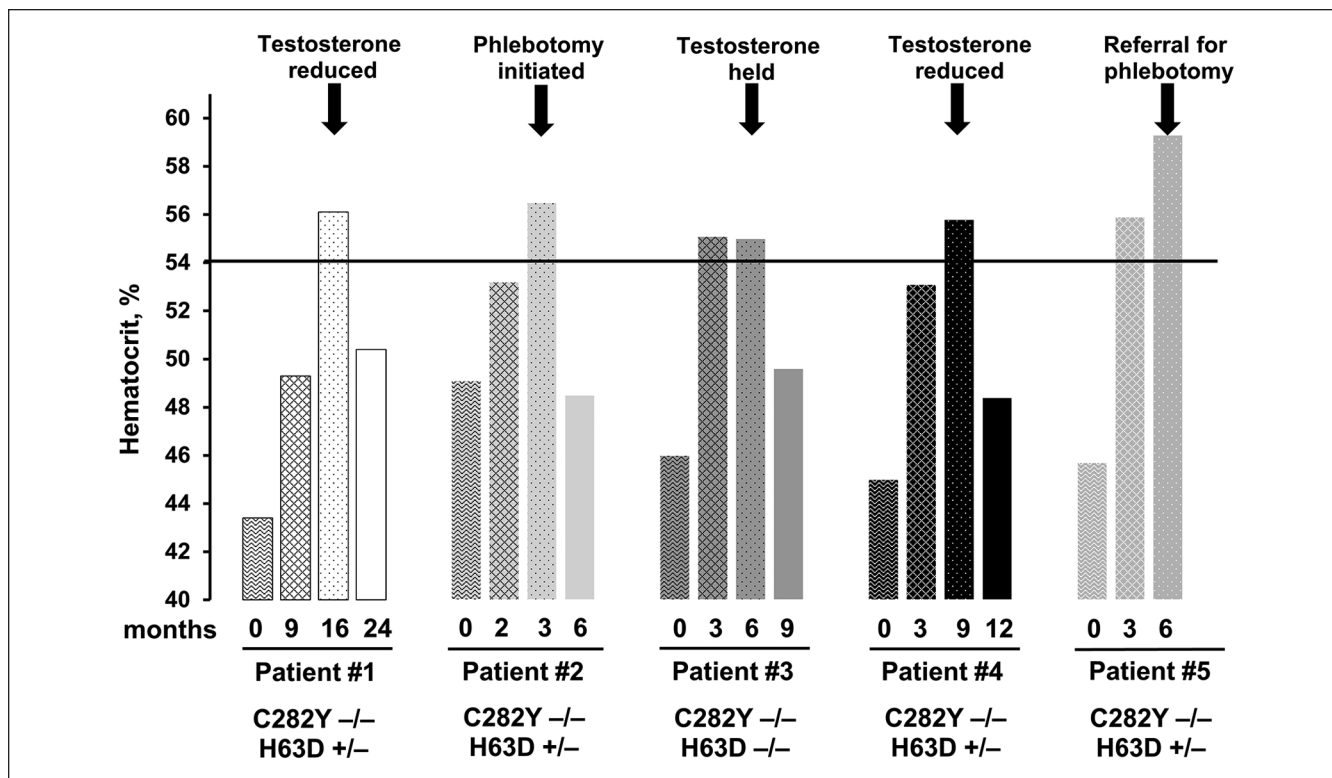
Figure 1. Hematocrit trends in study patients before and after sodium-glucose cotransporter-2 inhibitor initiation.

the decision was made to hold TRT and continue empagliflozin which resulted in early Hct improvements (Figure 1). As we were unable to identify other clinical or biochemical factors triggering erythrocytosis except initiation of empagliflozin, we requested genetic analysis of common mutations in the HFE gene which revealed that the patient was C282Y –/– and heterozygous for the H63D mutation. While being off TRT, he has developed hypogonadal symptoms; upon further discussions with the patient, TRT was resumed per initial prescription, and he was referred to hematology clinic for scheduled phlebotomies. The patient has had normal Hct trends since the referral to hematology clinic.

Patient 3 was a 63-year-old WM with past medical history of T2D, hypertension, dyslipidemia, and hypogonadism who presented to endocrinology clinic in June 2019 for optimization of diabetes management (Table 1). At the time of evaluation, his hypogonadism was optimally managed by topical testosterone preparation initially started in 2011. To optimize glycemic control, we initiated empagliflozin 10 mg daily. He was seen back in clinic 3 months later when routine evaluation showed improving A_{1c} at 7.4% and elevated Hct of 55.1%. At that point, we have discussed with the patient potential strategies to address new diagnosis of erythrocytosis. He decided to initially reduce TRT intensity by 50%; however, as his erythrocytosis persisted, he subsequently completely stopped TRT while continuing empagliflozin

therapy which resulted in the normalization of Hct level (Figure 1). As we were again unable to identify other clinical or biochemical factors triggering erythrocytosis except initiation of empagliflozin, we requested genetic analysis of common mutations in the HFE gene which did not identify C282Y or H63D pathogenic variants. The patient elected to remain off TRT while continuing empagliflozin therapy and since then his follow-up Hct levels remained within normal range.

Patient 4 was a 63-year-old WM with past medical history of T2D, OSA, hypertension, dyslipidemia, and hypogonadism who presented to endocrinology clinic in January 2020 for optimization of diabetes management (Table 1). His hypogonadism was optimally managed by injectable testosterone preparation which was initially prescribed about 9 months prior to his clinic visit. To optimize glycemic control, empagliflozin 10 mg daily was initiated and in 3 months uptitrated to 25 mg. Four months later, routine follow-up in endocrinology clinic demonstrated improvement in hemoglobin A_{1c} to 7.7%; however, he was also noted to have developed erythrocytosis. Following the discussion with the patient, the decision was made to hold TRT and continue empagliflozin which resulted in Hct normalization (Figure 1). As his sleep apnea was treated optimally and not being able to identify other clinical or biochemical factors triggering erythrocytosis except initiation of empagliflozin, we requested genetic analysis of common

mutations in the HFE gene which revealed that the patient was C282Y –/– and heterozygous for the H63D mutation. The patient decided to hold TRT while continuing empagliflozin therapy and since then his follow-up Hct levels remained within normal range.

Patient 5 was a 56-year-old WM with past medical history of T2D, CHF, chronic kidney disease, hypertension, dyslipidemia, and hypogonadism who was seen in clinic in July 2021 for optimization of diabetes management (Table 1). At that time, his hypogonadism was optimally managed by topical testosterone preparation initially started in 2014. To optimize glycemic control, empagliflozin 10 mg daily was prescribed. He was seen again in clinic in 3 months when routine evaluation showed elevated Hct of 55.9% (Figure 1). Following the discussion of risks associated with untreated erythrocytosis and having advised the patient to de-intensify TRT or stop empagliflozin, he insisted on continuing both treatments unchanged. He was seen again in clinic 3 months later, when his Hct was noted to be persistently elevated at 59.3%. As we were again unable to identify other clinical or biochemical factors triggering erythrocytosis except initiation of empagliflozin, we requested genetic analysis of common mutations in the HFE gene which revealed that the patient was C282Y –/– and heterozygous for the H63D mutation. The patient elected to continue both TRT to avoid the development of hypogonadal symptoms if testosterone prescription is stopped and empagliflozin for glycemic benefits. Therefore, he was referred to hematology clinic to initiate therapeutic phlebotomy.

Discussion

For the first time, this case series demonstrated that patients who develop erythrocytosis while prescribed both TRT and an SGLT-2 inhibitor may have risk of underlying heterozygosity in H63D variant of the HFE gene. Four of the 5 patients presented here had the HFE gene allele that can be encountered in up to 20% of US non-Hispanic whites.[14] Our study does support initial clinical observation that persons on stable TRT can develop erythrocytosis following SGLT-2 inhibitor initiation.[7] We believe that this safety concern has pathophysiological rationale since the cellular mechanisms by which testosterone and SGLT-2i can increase erythrocytes' production appear to be synergistic.

In our practice, we monitor Hct levels in men with hypogonadism on stable TRT during each routine clinic visit. Having found new-onset erythrocytosis in the study patients, we have initially excluded common causes of secondary erythrocytosis such as hypoxia from lung diseases or current smoking, advanced heart or renal disease, or exogenous erythropoietin use.[8,15] While TRT is known to cause erythrocytosis,[9,16] our patients were on stable regimens and had no instances of pre-empagliflozin elevation of Hct >54% suggesting that testosterone prescription was safe. After the addition of SGLT-2i, the rise in Hct in the study patients was not unexpected considering recent publication,[7] and in the absence of other factors that may predispose to erythrocytosis as per above, we decided to screen these persons for an HFE gene mutation.

The HFE gene mutations are the most common causes of HH, with homozygosity of the missense C282Y mutation most frequently resulting in clinical manifestations of iron overload. The H63D HFE mutation is a histidine-to-aspartic acid substitution at amino acid position 63 and is unlikely to be associated with clinical HH.[13] HFE is an upstream regulator of hepcidin, which is a protein that becomes upregulated during conditions of iron excess to reduce iron absorption in the gut. When the HFE gene mutation(s) is present, decreased hepcidin function leads to uninhibited iron gut absorption which may result in several biochemical and clinical alterations with excess red blood production being one of the manifestations.[11] The H63D homozygosity can also lead to HH; however, clinical penetrance of this mutation is much lower than in those who have homozygosity in the C282Y mutation.[11,17] Furthermore, previous studies have demonstrated that individuals with heterozygosity for H63D gene will have normal to slightly elevated biochemical indices of iron overload compared with wild-type individuals[18,19] or may tend to have higher baseline hemoglobin levels when compared with persons with C282Y or compound C282Y/H63D heterozygosity.[20] In addition, it was previously shown that high-volume transfusion donors (super donors) who had the H63D heterozygosity were characterized by significant reduction in hepcidin production compared with the wild-type super donors.[21]

On the contrary, both testosterone and SGLT-2i in *in vivo* and clinical studies have been shown to suppress hepcidin and increase erythropoietin levels thus promoting environment stimulating hematopoiesis and erythrocytosis.[16,22-25] The mechanisms behind SGLT2i-assiacted increase in Hct had been initially thought to be related to a hemoconcentration.[1,2] However, recently, SGLT-2i empagliflozin and dapagliflozin have been demonstrated to suppress hepcidin via pathways reminiscent to the testosterone effects and alter cellular metabolism promoting hypoxia which could, in turn, increase erythropoietin production.[4,22,25] Neither TRT nor SGLT-2 inhibitors alone have been shown to increase risk or thromboembolic events though theoretical concerns related to the effects from excessive testosterone prescription cannot be entirely dismissed.[9] We hypothesize that in the individuals who have heterozygous mutation in the HFE gene, the combined use of testosterone therapy and SGLT-2i may unmask predisposition to iron overload by further suppressing hepcidin activity and increasing hematopoiesis thereby placing these individuals at a risk of erythrocytosis. We have indirectly supported above concept by demonstrating that *de novo* erythrocytosis in these patients can be reversed if testosterone regimen has been discontinued or decreased.

This study has limitations. The one patient who did not have either HFE gene variants could still have a genetic

predisposition to develop iron overload due to rare genetic mutation(s) that can affect different steps in iron regulation[11,17]; however, these mutations are not routinely tested for in commercial laboratories. These rarer mutations are also more likely to present earlier in life than mutations in the HFE gene.[26] Another limitation is the retrospective design of this case series when most of the patients were found to have the H63D allele after developing erythrocytosis. The method of reducing Hct in our patients was not uniform with different options considered from stopping or reducing TRT or discontinuing SGLT-2i to referral to therapeutic phlebotomy; however, following the discussion with each patient of potential mitigation strategies, the final decision was tailored to the patients` wishes. There are no prior studies or clear recommendations to guide decision-making in such situations, so the approaches to reduce Hct until then should be patient-centric. Iron studies were not addressed in patients before or after the development of erythrocytosis due to either lack of indications to measure the indices as red blood were normal in former situation or retrospective nature of the study in the latter scenario. Finally, all our patients were non-Hispanic whites, and it is unclear if the study results can be replicated in persons of other ethnic and racial background.

Conclusion

Our study confirms that risk new-onset erythrocytosis in men with hypogonadism on stable testosterone therapy following the initiation of SGLT-2i does exist and should not be dismissed. For the first time, we report here that presence of the H63D allele in the HFE gene may support the development of secondary erythrocytosis in these patients. We therefore recommend that patients on combined TRT and SGLT-2i should be monitored for new-onset erythrocytosis. Also, patients who developed erythrocytosis can be offered genetic screening for common mutations in the HFE gene to potentially expand the management options. Periodic therapeutic phlebotomy can be considered if HFE gene allele is detected to allow continuation of pharmacological management of T2D and hypogonadism. At minimum, options for these patients should include either de-escalation of TRT or SGLT-2i therapy with a goal to normalize Hct and eliminate risk of hyperviscosity.

Author Contributions

ARG drafted the manuscript. KAS and ARG researched data and wrote the manuscript.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: ARG is an employee of the US Department of Veterans Affairs and his opinions expressed in this paper are those of the authors and do not represent the views of the Department of Veterans Affairs or the US Government. ARG has received research support from AbbVie paid to the institution. KAS has no competing interests.

Ethics Approval

Our institution does not require ethical approval for reporting individual cases or case series.

Informed Consent

Verbal informed consent was obtained from the patient for their anonymized information to be published in this article.

ORCID iDs

Kamilya A. Schumacher (iD) https://orcid.org/0000-0002-2858-5202
Aidar R. Gosmanov (iD) https://orcid.org/0000-0002-4197-5344

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