Effects of empagliflozin on erythropoiesis in patients with type 2 diabetes: Data from a randomized, placebo-controlled study

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Abstract
Sodium-glucose cotransporter-2 (SGLT2) inhibitors have been shown to significantly reduce hospitalization for heart failure (HHF) and cardiovascular (CV) mortality in various CV outcome trials in patients with and without type 2 diabetes mellitus (T2D). SGLT2 inhibition further increased haemoglobin and haematocrit levels by an as yet unknown mechanism, and this increase has been shown to be an independent predictor of the CV benefit of these agents, for example, in the EMPA-REG OUTCOME trial. The present analysis of the EMPA haemodynamic study examined the early and delayed effects of empagliflozin treatment on haemoglobin and haematocrit levels, in addition to measures of erythropoiesis and iron metabolism, to better understand the underlying mechanisms. In this prospective, placebo-controlled, double-blind, randomized, two-arm parallel, interventional and exploratory study, 44 patients with T2D were randomized into two groups and received empagliflozin 10 mg or placebo for a period of 3 months in addition to their concomitant medication. Blood and urine was collected at baseline, on Day 1, on Day 3 and after 3 months of treatment to investigate effects on haematological variables, erythropoietin concentrations and indices of iron stores. Baseline characteristics were comparable in the empagliflozin (n = 20) and placebo (n = 22) group. Empagliflozin led to a significant increase in urinary glucose excretion (baseline: 7.3 ± 22.7 g/24 h; Day 1: 48.4 ± 34.7 g/24 h; P < 0.001) as well as urinary volume (baseline: 1740 ± 601 mL/24 h; Day 1: 2112 ± 837 mL/24 h; P = 0.011) already after 1 day and throughout the 3-month study period, while haematocrit and haemoglobin were only increased after 3 months of treatment (haematocrit: baseline: 40.6% ± 4.6%; Month 3: 42.2% ± 4.8%, P < 0.001; haemoglobin: baseline: 136 ± 19 g/L; Month 3: 142 ± 25 g/L; P = 0.008). In addition, after 3 months, empagliflozin further increased red blood cell count (P < 0.001) and transferrin concentrations (P = 0.063) and there was a trend toward increased erythropoietin levels (P = 0.117), while ferritin (P = 0.017), total iron (P = 0.053) and transferrin saturation levels (P = 0.030) decreased. Interestingly, the increase in
Sodium-glucose cotransporter-2 (SGLT2) inhibitors are glucose-lowering drugs currently used to treat patients with type 2 diabetes mellitus (T2D). These agents act by inhibiting SGLT2 in the proximal tubule of the kidney, with a subsequent increase in urinary glucose excretion, thus lowering blood glucose levels. Several placebo-controlled cardiovascular outcome trials (CVOTs) with SGLT2 inhibitors (EMPA-REG OUTCOME with empagliflozin,3 the CANVAS programme2 and CRESCENDO with canaglifozin, DECLARE with dapaglifozin4) demonstrated a reduction in cardiovascular (CV) events as well as a reduction in hospitalization for heart failure (HHF) in patients with diabetes.5 In addition empagliflozin and dapaglifozin were recently found to reduce heart failure (HF) events in patients with HF with reduced ejection fraction, with and without diabetes.5,6 The underlying mechanisms of these beneficial effects of SGLT2 inhibitors on HF-related events remain unclear. Additionally, all CVOTs showed an increase in haematocrit and haemoglobin levels in response to SGLT2 inhibition and these changes appeared to best predict CV death reduction in a mediation analysis of the EMPA-REG OUTCOME trial.3 The increase in haemoglobin and haematocrit concentrations seems attributable to an increase in erythropoietin (EPO) levels and stimulated erythropoiesis.7 Several hypotheses have been raised to explain this effect on EPO production, including an increase in renal tissue oxygen delivery by activation of tubuloglomerular feedback through enhanced sodium detection at the distal renal tubules by the juxtaglomerular apparatus, or a direct stimulation of EPO by ß-Hydroxybutyrate.8 In addition, SGLT2 inhibitor-induced glucosuria could reduce ATP consumption by the Na/K pump in proximal tubular epithelial cells, thus leading to improvement in hypoxia and inflammation in the microenvironment around the proximal tubules, with subsequent reversion of myofibroblasts to EPO-producing fibroblasts.9 However, the exact mechanisms contributing to the increase in EPO resulting from SGLT2 inhibitor treatment remain unexplored; therefore, the present post hoc analysis of the EMPA haemodynamics study (EudraCT Number: 2016-000172-19),10 a prospective, placebo-controlled, double-blind, randomized, two-arm parallel, exploratory study in patients with T2DM, examined the different potential mechanisms.

2 | METHODS

In the present study, performed at the University of Aachen, 44 patients with T2D were randomized to receive empagliflozin 10 mg or placebo for 3 months in addition to their concomitant medication (Figure S1). The primary endpoint was the effect of empagliflozin on haemodynamic characteristics, and, as secondary endpoints, we analysed changes in blood and urine variables, as well as changes in echocardiographic variables. For this post hoc analysis, blood and urine were collected at baseline, on Day 1, on Day 3 and after 3 months of treatment to investigate the effects on haematological variables, erythropoietin concentrations and indices of iron stores. Outcome variables were analysed using linear mixed models with fixed effects for treatment, visits (Day 1, Day 3 and 3 months) and baseline measurement of the variable. Data analysis was performed in 36 patients because two patients were excluded for protocol violations, three patients did not participate at Visit 5 after 3 months, and three patients were excluded from the data analysis because of missing urine values. Baseline characteristics were comparable in both groups: mean age 62 ± 6.8 years, 81% male, mean glycated haemoglobin concentration 60.7 mmol/mol ± 11.5 mmol/mol, mean body mass index 31.3 ± 4.6 kg/m², mean estimated glomerular filtration rate 83 ± 19 mL/min/1.73 m², history of CV in 71%, and presence of HF in 43% of all patients. Patients had a mean (SD) baseline blood pressure of 135/81 (16.9/13.2) mmHg. Baseline medication, including antidiabetic drugs, renin-angiotensin-aldosterone system inhibition, beta blockers and statins, did not differ between the two groups (Figure S2).

The sample size calculation was conducted based on a repeated-measure analysis of variance of the primary endpoint, including baseline and three repeated measures, two treatment levels, and a treatment-by-time interaction tested using an F-test. Outcome variables were analysed using linear mixed models with fixed effects for treatment, visit (Day 1, Day 3 and 3 months) and baseline measurement of the variable. Values are mean ± SD for normally distributed
data and median and interquartile range for non-normally distributed data; P values at baseline were calculated using t-tests and
P values for the intervention effect at Day 1, Day 3 and Month 3 were calculated using the Wald method. Correlations between changes from baseline at different timepoints were calculated for selected variables using the Spearman rho correlation coefficient.

3 | RESULTS

Empagliflozin treatment in the present study had no significant effect on haemodynamic variables after 1 or 3 days, nor after 3 months, but led to rapid and sustained significant improvement of diastolic function. As expected, empagliflozin treatment significantly increased urinary glucose excretion already after 1 day from 7.3 ± 22.7 to 48.4 ± 34.7 g/24 h (P < 0.001). Urinary volume significantly expanded in parallel with glucosuria after Day 1 from 1740 ± 601 to 2112 ± 837 mL/24 h (P = 0.011) and remained significantly increased after 3 months of treatment (2319 ± 873 mL/24 h; P = 0.001) compared to placebo (Figures S3 and S4). In addition, empagliflozin treatment led to a significant increase in haematocrit and haemoglobin levels after 3 months (haemoglobin: baseline: 136 ± 19 g/L; Month 3: 142 ± 25 g/L; P = 0.008). Empagliflozin increased red blood cell count (P < 0.001), erythropoietin levels (P = 0.117) and transferrin concentrations (P = 0.063) while decreasing ferritin (P = 0.017), total iron (P = 0.053) and transferrin saturation (P = 0.030) only after 3 months of treatment (Figure 1A). To further explore the potential mechanisms involved in empagliflozin-induced EPO production we performed additional analyses (Figures S5). As shown in Figure 1B and C, changes in EPO levels did not correlate with changes in urinary sodium excretion after 3 days or 3 months of empagliflozin treatment, making natriuresis with subsequent activation of tubuloglomerular feedback an unlikely explanation for the increase in EPO. In addition, we did not observe a significant correlation of changes in β-Hydroxybutyrate with changes in EPO levels. However, changes in urinary glucose excretion strongly correlated with changes of erythropoietin in empagliflozin-treated patients at the 3-month timepoint (Spearman rho 0.64, P = 0.008; Figure 1B,C).

4 | DISCUSSION

In this randomized, placebo-controlled, double-blind study in patients with T2D and prevalent atherosclerotic CVD or high CV risk,
resembling the populations studied in CVOTs with SGLT2 inhibitors, empagliflozin led to a significant increase in haematocrit and haemoglobin levels after 3 months, an effect not yet present after 1 or 3 days of treatment, suggesting that haemoconcentration might not be the main mechanism leading to the increase in these variables. However, others have reported that SGLT2 inhibition simultaneously reduced extracellular and plasma volume; consequently, we cannot exclude a more delayed occurrence of blood volume reduction contributing to the observed increase in haematocrit at the 3-month time point in our study. Interestingly, the relevance of volume unloading for the therapeutic efficacy of SGLT2 inhibition was recently challenged by post hoc analysis of the EMPEROR-Reduced Trial, which showed comparable therapeutic efficacy of empagliflozin in patients with and without recent volume overload.

As an alternative mechanism, we detected stimulated erythropoiesis with augmented iron utilization as shown by increased erythropoietin levels and appropriate changes in iron measures. This suggests that the increase in EPO levels might be attributable to reduced tubular glucose reabsorption in response to SGLT2 inhibition, possibly resulting in diminished cellular stress, as a potential mechanism for increased renal erythropoietin secretion. However, this observation does not imply causality and we cannot exclude the possibility that other factors directly or indirectly regulated by SGLT2 inhibition are of relevance in this context. This might include modulation of metabolic pathways favouring a fasting-like response with induction of ketone bodies and catabolism of free fatty acids and branched chain amino acids. Further, modulation of erythropoiesis might relate to inhibition of inflammatory pathways as a potential mechanism for improved EPO production.

The present analysis has certain limitations: our data provide an exploratory finding in a limited number of patients and warrant confirmation in a larger study, with changes in haematological variables and erythropoiesis defined as primary outcomes. Furthermore, 81% of study participants were male, precluding sex-specific analysis of treatment response to SGLT2 inhibition. In addition, the study included only few patients with chronic kidney disease, a population known to exhibit impaired erythropoietin production. Additional studies are warranted to extend our findings to these populations. Nevertheless, the present study was randomized, blinded and placebo-controlled, and changes in blood and urine levels were predefined exploratory endpoints.

In summary, our data showing a strong correlation between empagliflozin-induced glucosuria and the increase in erythropoietin levels in T2DM bolster the hypothesis that SGLT2 inhibitors could limit metabolic and oxidative stress in the microenvironment around the proximal tubules with subsequent reversion of myofibroblasts to EPO-producing fibroblasts. This interpretation is limited, however, by the small sample size of the present study and additional investigation of the kidney microenvironment in response to SGLT2 inhibition is required.

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CONFLICT OF INTEREST
K.T., M.R., N.U.K.H., J.M. and A.K. report no potential conflict of interest. J.J. has given lectures for Bayer and Fresenius Medica Care. In addition, he holds four patents related to the topic of the manuscript and is inventor of an additional, already-sold patent to Baxter. M.B. received speaker honoraria from Astra-Zeneca and Boehringer-Ingelheim and was national coordinator of DAPA-HF and EMPEROR-Reduced. N.M. has received support for clinical trial leadership from Boehringer Ingelheim and Novo Nordisk, served as a consultant to Boehringer Ingelheim, Merck, Novo Nordisk, AstraZeneca and BMS, received grant support from Boehringer Ingelheim, Merck, Novo Nordisk, and served as a speaker for Boehringer Ingelheim, Merck, Novo Nordisk, Lilly, BMS and Astra Zeneca. M.L. received grants and personal fees from Boehringer Ingelheim, MSD and Novo Nordisk, and personal fees from Amgen, Sanofi, Astra Zeneca, Bayer and Lilly.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14517.

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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