

Endothelial and oxidative biomarker profiles in atrial fibrillation according to SGLT2 inhibitor exposure: a cross-sectional study

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Article

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Abstract

Background

Sodium-glucose co-transporter 2 (SGLT2) inhibitors improve outcomes in heart failure and chronic kidney disease, but their vascular effects in atrial fibrillation remain incompletely characterized. We assessed whether SGLT2 inhibitor use is associated with endothelial and oxidative biomarker profiles and whether empagliflozin and dapagliflozin differ in this respect.

Methods

In this single-center cross-sectional study with prospective recruitment, 287 patients with non-valvular atrial fibrillation receiving long-term oral anticoagulation were included. Six biomarkers were assigned a priori to endothelial, oxidative and integrative domains. Patients receiving an SGLT2 inhibitor (n = 168) were compared with those not receiving one (n = 119), and empagliflozin (n = 98) with dapagliflozin (n = 70). Associations were assessed using multiple regression adjusted for age, sex, eGFR, type 2 diabetes, chronic heart failure, left ventricular ejection fraction, left atrial dimension and coronary artery disease, with Holm correction across the six panel markers.

Results

In crude analyses, SGLT2 inhibitor use was associated with higher sVCAM-1 and sE-selectin and lower angiopoietin-2 and GDF-15 concentrations, while 8-OHdG and advanced oxidation protein products did not differ. After multivariable adjustment, only sVCAM-1 remained associated with SGLT2 inhibitor use (+ 12.8%; 95% CI + 3.6 to + 22.9; p = 0.006; Holm-adjusted p = 0.036). Differences in NT-proBNP and high-sensitivity troponin T observed in crude analyses disappeared after adjustment. Empagliflozin and dapagliflozin did not differ across any of the eight analytes measured; the comparison had 80% power to detect a difference of approximately 0.44 standard deviations.

Conclusions

In patients with non-valvular atrial fibrillation, endothelial but not oxidative biomarker profiles differed according to SGLT2 inhibitor exposure, although only sVCAM-1 remained independently associated after adjustment. Empagliflozin and dapagliflozin were not statistically distinguishable within the resolution of this study. These cross-sectional findings do not establish causality and support prospective evaluation of sVCAM-1 as a marker of endothelial heterogeneity.

Research insights

What is currently known about this topic?	• SGLT2 inhibitors improve outcomes in heart failure and chronic kidney disease. • Their vascular biomarker profile in atrial fibrillation remains poorly defined.
What is the key research question?	Do biomarkers differ by SGLT2 inhibitor use or molecule in patients with atrial fibrillation?
What is new?	• Only sVCAM-1 remained associated with SGLT2 inhibitor use after full adjustment. • Oxidative DNA and protein damage markers did not differ by SGLT2 inhibitor use. • No analyte differed detectably between empagliflozin and dapagliflozin.
How might this study influence clinical practice?	• The findings do not support biomarker-guided selection between empagliflozin and dapagliflozin.

1. Introduction

After the cardiovascular benefit of empagliflozin had been demonstrated in patients with type 2 diabetes and established cardiovascular disease in the EMPA-REG OUTCOME trial [1], empagliflozin and dapagliflozin showed clinical benefit across the whole spectrum of heart failure [2–5] and in chronic kidney disease [6, 7]. According to the 2026 ESC guidelines, dapagliflozin or empagliflozin is recommended in patients with symptomatic heart failure irrespective of ejection fraction, in order to reduce the risk of hospitalization for heart failure or of cardiovascular death [8]. In most patients with chronic kidney disease, in turn, an SGLT2 inhibitor is recommended to be added to renin-angiotensin system blockade in order to limit the risk of progression of kidney disease and cardiovascular risk [9]. Because obesity, type 2 diabetes and chronic kidney disease remain closely linked with cardiovascular disease, including heart failure, within the cardiovascular-kidney-metabolic continuum [10], drugs acting simultaneously on several of its components occupy a particular place in practice. In this group of patients, treatment decisions rest primarily on clinical assessment; whether, and to what extent, measurements of circulating biomarkers can support them remains an open question.

Several routes have been proposed through which SGLT2 inhibition may act on the vessel wall. At the level of the endothelial cell, a reduction in the generation of reactive oxygen species and, in experimental studies, preservation of the integrity of the glycocalyx have been described [12]. A second proposed route is modulation of the function of sodium-hydrogen exchanger 1 (NHE1), although the evidence for it remains disputed [11]. This premise nevertheless bears on the arrhythmia studied here. In right atrial appendage biopsies obtained during cardiac surgery, expression of this exchanger was higher in patients with heart failure with preserved ejection fraction and atrial fibrillation than in patients with the same diagnosis but in sinus rhythm. In isolated human atrial cardiomyocytes, empagliflozin inhibited its activity to a degree similar to that of the selective inhibitor cariporide. Both observations, however, come from very small subgroups, and in patients with an ejection fraction below 40% the expression of the

exchanger was, in that same work, lower than in the remaining groups [13]. The preponderance of the data supporting these mechanisms comes from experimental models, from cell culture and from a small number of studies on isolated human tissues conducted by indirect methods [11, 12], or from cohorts selected on the basis of diabetes rather than on the basis of the arrhythmia. A divergence between functional and biomarker evidence has, moreover, also been described in humans. A randomized trial enrolled patients with type 2 diabetes at high cardiovascular risk, most of them with subclinical carotid atherosclerosis. Twelve weeks of dapagliflozin at a dose of 10 mg daily improved flow-mediated dilation of the brachial artery relative to glibenclamide and with matched glycemic control, whereas concentrations of the soluble adhesion molecules VCAM-1 and ICAM-1 and of free 8-isoprostane did not differ between the groups either before or after treatment [14]. It has not been established, however, to what extent these mechanisms are detectable in circulating biomarkers in patients with atrial fibrillation – that is, in cohorts defined by the arrhythmia rather than by diabetes.

No single analyte, moreover, captures endothelial biology. The soluble adhesion molecule VCAM-1, also known as CD106, is a cell-surface protein of the immunoglobulin superfamily that mediates the adhesion and transmigration of leukocytes from the circulation into tissue through binding of the $\alpha 4\beta 1$ integrin, and its soluble form arises from shedding of the extracellular domain by the metalloproteinase ADAM-17 [15]. E-selectin, expressed exclusively on endothelial cells and induced by proinflammatory cytokines, corresponds instead to an earlier and partly separate step of the same pathway. By binding ligands on circulating leukocytes, it captures and slows them, which is what makes integrin activation, firm adhesion and transmigration possible. Its soluble form arises from shedding of the membrane-bound form and serves as a surrogate indicator of endothelial activation [16]. Angiopoietin-2, by contrast, acts outside the adhesion molecule system: it is a context-dependent ligand of the Tie2 receptor that can oppose the vessel-stabilizing action of angiopoietin-1; when Tie2 signaling is attenuated, it can activate the $\beta 1$ integrin, leading to reorganization of intercellular junctions and to a reduction in surface vascular endothelial cadherin [17]. Oxidative damage likewise takes several forms. 8-hydroxy-2'-deoxyguanosine, a product of the oxidation of deoxyguanosine, is regarded as an important biomarker of oxidative DNA damage and is measured in urine, blood, tissue and saliva [18]. Advanced oxidation protein products, in contrast, are related to oxidatively modified plasma proteins, particularly albumin, and their formation can be induced by oxidation of proteins with hypochlorous acid [19]. GDF-15, a cytokine of the transforming growth factor β superfamily, is strongly induced under conditions of stress and inflammation; in experimental models its increase has also been linked with myocardial injury and pressure overload [20]. For this reason it was treated in the present work as a marker integrating a broader biological burden rather than as a marker of a single pathophysiological pathway. Considering these analytes as three domains defined in advance, rather than as a list, makes it possible to pose the question at the level at which the underlying biology actually differs.

Atrial fibrillation is a particularly informative setting for such a question. Endothelial activation is documented in this arrhythmia: in a population-based cohort followed for twenty years, a higher concentration of the soluble molecule VCAM-1 was associated with incident atrial fibrillation and was, after adjustment for age and sex, the only one of thirteen inflammatory markers measured to retain this

association [21]. This observation was reproduced in a cohort of 48,495 individuals without a previous diagnosis of atrial fibrillation or heart failure, followed for almost fourteen years. A one standard deviation increase in sVCAM-1 concentration corresponded to a hazard ratio of 1.72 (95% CI 1.54–1.91) for incident atrial fibrillation and of 2.04 (95% CI 1.78–2.34) for incident heart failure. The association persisted both after adjustment for NT-proBNP and after adjustment for C-reactive protein, leukocyte count, tumor necrosis factor and interleukin 6 [22]. In patients with atrial fibrillation, SGLT2 inhibitors are used because of coexisting cardiometabolic indications and not as treatment of the arrhythmia itself [23]. Consistent with this interpretation, in a randomized trial in patients with persistent atrial fibrillation free of diabetes, heart failure and chronic kidney disease, three months of dapagliflozin after catheter ablation changed neither arrhythmia burden nor left atrial dimension [24]. A narrower question, of direct relevance to the individual choice of treatment, also remains open: empagliflozin and dapagliflozin have never been compared directly in a randomized trial, and juxtaposing the results of separate studies does not allow the molecule to be separated from the population in which it was assessed. The few direct comparisons available to date have covered natriuretic peptide prospectively or, in observational cohorts, hard endpoints; markers of endothelial activation and of oxidative stress have not been compared between the two molecules in any of them [25–27].

We therefore compared six markers assigned before analysis to three domains – endothelial, oxidative and integrative – in patients with atrial fibrillation receiving and not receiving an SGLT2 inhibitor, taking the endothelial domain as the primary endpoint. The secondary comparison, planned before analysis, was the direct comparison of the two molecules in use. In addition to the panel, NT-proBNP and high-sensitivity troponin T were measured as clinical reference markers of cardiovascular disease burden. The study was conducted in a single cohort, with uniform eligibility criteria, a single pre-analytical protocol and blood sampling standardized with respect to the dosing interval rather than to the time of day alone. The design is cross-sectional and observational, and all results are therefore presented as associations.

2. Materials and methods

2.1. Study design and population

The study was cross-sectional, with prospective recruitment and a single collection of material from each participant. It was conducted at a single center, in the Department of Cardiology, Clinical Pharmacology and Internal Medicine of the Dr Jan Biziel University Hospital No. 2 in Bydgoszcz, between 9 January 2025 and 30 March 2026. Patients were recruited through two pathways. In both pathways, consecutive patients meeting the eligibility criteria were enrolled continuously. The first was the Heart Failure Outpatient Clinic, which provides post-discharge care and long-term outpatient care for patients previously treated in this Department (n = 147). The second was admission for elective catheter ablation of atrial fibrillation (n = 140). Patients from the ablation pathway were admitted electively, from a waiting list, the waiting time for the procedure not infrequently amounting to several months; at the time of blood sampling they were in a stable outpatient condition, before the procedure and outside any period of

exacerbation. No blood was drawn under peri-procedural conditions or during hospitalization for acute causes. No register of screened patients was kept, and it is therefore not possible to state the number of patients assessed for eligibility or the reasons for non-inclusion.

Both pathways were conducted within the same unit, with identical inclusion and exclusion criteria and an identical pre-analytical protocol. On this basis, the cohort of 287 patients was analyzed together as a single population. The pathways did, however, differ in the frequency of chronic heart failure: it was present in all 147 patients recruited in the Heart Failure Outpatient Clinic and in 49 of the 140 patients from the ablation pathway. Chronic heart failure, diagnosed before enrollment in accordance with the ESC guidelines in force during the recruitment period [28], was present in a total of 196 patients (68.3%) and was a feature characterizing the population rather than a condition of participation in the study. The structure of the cohort and both planned comparisons are shown in Fig. 1.

The cohort included 287 adults with electrocardiographically confirmed non-valvular atrial fibrillation receiving long-term oral anticoagulation. Patients were recruited between 9 January 2025 and 30 March 2026 through the Heart Failure Outpatient Clinic (n = 147) or before elective atrial fibrillation ablation (n = 140), using identical eligibility and pre-analytical procedures.

SGLT2 inhibitor exposure required documented uninterrupted treatment with empagliflozin or dapagliflozin 10 mg once daily for at least 3 months. Blood was collected between 07:00 and 09:00 before the morning dose. The primary comparison was SGLT2 inhibitor use (n = 168) versus no use (n = 119); the secondary comparison was empagliflozin (n = 98) versus dapagliflozin (n = 70). Chronic heart failure was present in 196 patients but was not required for inclusion.

All six panel biomarkers were available for all participants. NT-proBNP and hs-TnT were unavailable in two untreated patients, so analyses of these markers included 285 participants. No imputation was used. Arrows denote analytical contrasts rather than temporal direction. The figure describes the enrolled cohort rather than screening flow because screened patients were not recorded; box sizes are not proportional. Abbreviations: hs-TnT, high-sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SGLT2, sodium-glucose co-transporter 2.

2.2. Inclusion and exclusion criteria

The inclusion criteria comprised an age of at least 18 years and clinically diagnosed atrial fibrillation confirmed electrocardiographically in accordance with the 2024 ESC/EACTS guidelines [23], in the absence of a mechanical valve prosthesis and of moderate or severe mitral stenosis. Treatment with a direct oral anticoagulant administered continuously for at least six months, including at least three months at an unchanged dose, or treatment with a vitamin K antagonist administered continuously for at least six months, with a target INR of 2.0–3.0 and a time in therapeutic range determined by the Rosendaal method of not less than 65%, was additionally required. Two further criteria were conditional and applied only to some of the patients. In patients with previously diagnosed chronic heart failure, clinically compensated and stable heart failure at the time of qualification was required: euolemia, no

need for intravenous therapy and stable oral heart failure therapy, including an unchanged dose, for at least three months, of the diuretic used because of heart failure. In patients receiving an SGLT2 inhibitor, treatment with empagliflozin or dapagliflozin administered continuously for at least three months before enrollment was required; patients treated for a shorter period were not eligible. Neither the presence of chronic heart failure nor the use of an SGLT2 inhibitor was therefore a condition of participation in the study; both of these features — where present — did, however, entail additional eligibility requirements.

The exclusion criteria comprised acute conditions, comorbidities, laboratory thresholds and treatment in use. Among acute conditions, active infection or sepsis and fever of unknown etiology were exclusions, as were, within the three months preceding qualification, shock of any cause, acute coronary syndrome or stroke, heart failure decompensation, surgery, and bleeding requiring intervention or transfusion; heart failure decompensation was defined as worsening of symptoms requiring hospitalization, treatment in an emergency department or an urgent unscheduled visit with intensification of therapy. Among comorbidities, exclusions comprised severe anemia (hemoglobin concentration below 8 g/dl), active malignancy, autoimmune disease requiring steroid or immunosuppressive therapy, uncontrolled hyperthyroidism or hypothyroidism, liver cirrhosis, chronic inflammatory bowel disease, decompensated diabetes requiring intravenous insulin therapy, and alcohol dependence syndrome. The laboratory criteria comprised impaired liver function, defined as an INR above 1.5 in patients not receiving a vitamin K antagonist, a total bilirubin concentration above 1.5 mg/dl, or aspartate or alanine aminotransferase activity above three times the upper limit of normal, and an eGFR below 15 ml/min/1.73 m² on the day of qualification. On account of treatment in use, the use of glucocorticoids, non-steroidal anti-inflammatory drugs, heparin or hormone replacement therapy within the four weeks preceding qualification and antiplatelet treatment within three months were exclusions. Mental disorders that could threaten the reliability of the results were a further exclusion.

2.3. Definition of exposure

Exposure was defined as documented, continuous treatment with empagliflozin or dapagliflozin ongoing at the time of blood sampling and administered without interruption for at least three months. The exposed group therefore comprised only patients already on treatment, in whom therapy had been started before enrollment in the study; the study did not include follow-up from the time therapy was started. The primary comparison was that of patients receiving an SGLT2 inhibitor (n = 168) with those not receiving one (n = 119). The secondary comparison, planned before analysis, was the direct comparison of empagliflozin (n = 98) with dapagliflozin (n = 70).

All patients receiving an SGLT2 inhibitor were given it at the same daily dose of 10 mg, taken in the morning. Patients receiving an SGLT2 inhibitor at another daily dose were not eligible for the study. Blood was drawn within a 07:00–09:00 window, before the morning dose was taken, and therefore at the end of the dosing interval, approximately twenty-four hours after the preceding dose. Sampling thus corresponded to pre-dose conditions both for the oral anticoagulant and for the SGLT2 inhibitor.

2.4. Blood sampling and plasma preparation

Blood sampling, plasma processing, aliquoting and storage were carried out in all participants using the same materials, the same equipment and the same standard operating procedure. This applied equally to both recruitment pathways and to patients receiving and not receiving an SGLT2 inhibitor; neither of these groups was handled according to a different pre-analytical protocol.

Venous blood was obtained by venipuncture into 5 ml sodium heparin tubes (VACUETTE, Greiner Bio-One). Sampling was performed within a standardized morning window (07:00–09:00), in the fasting state, after an overnight interval without food intake. Each sample was inspected visually before measurement for hemolysis, lipemia and icteric discoloration; samples showing any of these features were to be excluded before measurement. None of these interferences was observed in the material studied, and no sample was excluded for this reason.

The material was processed within 60 minutes of sampling. Plasma was separated in a single centrifugation step at $2000 \times g$ for 15 minutes at 4°C, in accordance with the procedure for the preparation of platelet-poor plasma. The plasma fraction was collected without disturbing the cellular layer and divided into aliquots of at least 1 ml in polypropylene Eppendorf tubes. Aliquots were stored at – 80°C in a low-temperature freezer (MDF-DU500VH-PE; Panasonic Healthcare/PHCbi) until measurement; each of them underwent a single freeze-thaw cycle before measurement.

2.5. Biomarker measurements

The concentrations of the six panel biomarkers, assigned to the endothelial, oxidative and integrative domains, were measured by enzyme-linked immunosorbent assay using commercially available kits. 8-hydroxy-2'-deoxyguanosine and advanced oxidation protein products were measured with competitive inhibition assays, whereas sVCAM-1/CD106, sE-selectin/CD62E, angiopoietin-2 and GDF-15 were measured with sandwich assays. Manufacturers, catalog numbers, assay format and units are given in Table S1.

For each biomarker, plasma samples from all 287 participants were analyzed on 96-well plates. Samples were distributed at random across the plates in order to limit the possibility of systematic plate-related bias. On each plate a full calibration curve was generated independently using the standards supplied with the corresponding kit, and concentrations for the samples analyzed on a given plate were calculated from the curve obtained on that same plate. For each biomarker, the same pooled plasma control was placed on every plate, providing a common reference point for monitoring inter-plate variability.

Some of the samples were analyzed in technical replicates; these served to assess intra-assay precision and were not treated as independent biological observations. For all six panel assays the intra-assay coefficient of variation did not exceed 5% and the inter-assay coefficient of variation did not exceed 10%. The measurements were performed by two trained laboratory staff members, with all plates for a given biomarker handled by the same operator throughout the measurement series. Within any given analyte, operator identity could therefore neither differentiate samples nor generate a difference between the groups compared.

Absorbance was measured with a Thermo Scientific Varioskan LUX multimode reader (Thermo Fisher Scientific; serial number 3020–80651) operating in absorbance mode, at a wavelength of 450 nm. The instrument is equipped with a xenon flash lamp and a double monochromator for wavelength selection, with photomultiplier detection; the declared photometric accuracy is 0.003 Abs or $\pm 1\%$ over the 400–1000 nm range, and the linear measurement range is 0–4 Abs at 450 nm in the 96-well format. Data acquisition and initial processing were carried out in Thermo Scientific SkanIt Software v6.1. Because the panel combined competitive and sandwich assays, the calibration model indicated by the manufacturer for the given assay was used, rather than a single mathematical model imposed on all assays of differing analytical format.

2.6. Endpoint domains

Each of the six panel markers was assigned before analysis to exactly one of three domains: endothelial (sVCAM-1, sE-selectin, angiopoietin-2), oxidative (8-hydroxy-2'-deoxyguanosine, advanced oxidation protein products) and integrative (GDF-15). These assignments were not changed after the analysis had begun. The primary endpoint was the endothelial domain, derived from the mechanistic premise presented in the second paragraph of the Introduction; the oxidative domain and the integrative marker were analyzed as secondary endpoints. The correction for multiplicity was nevertheless applied to all six panel markers together, and therefore to a family broader than the primary domain, which makes it more conservative than the endpoint hierarchy adopted would require.

In addition to the panel, NT-proBNP and high-sensitivity troponin T were measured. These analytes were not assigned to any of the domains and were treated as clinical reference markers, serving to assess the extent to which the set of covariates adopted captures the cardiovascular disease burden differentiating the groups compared. Abolition after adjustment of the differences present in the crude comparison was taken as evidence that these variables capture a substantial part of that burden.

2.7. Statistical analysis

The analyses were performed in Statistica v. 13.3 (TIBCO Software Inc., Palo Alto, CA, USA). A two-sided significance level of 0.05 was adopted.

The rule for selecting descriptive statistics was adopted before analysis and applied uniformly to all continuous variables so that the form of description would correspond to the test used. Variables with an absolute value of skewness not greater than 1 are presented as mean $\pm$ standard deviation and were compared with the independent-samples Student t test; variables with skewness exceeding this value are presented as median [interquartile range] and were compared with the Mann-Whitney U test. For continuous variables, the form of description given in Tables 1–3 therefore indicates unambiguously the test used. This rule was departed from in favor of uniformity only for the biomarkers: the concentrations of all of them are presented as median [interquartile range] and were compared with the Mann-Whitney U test irrespective of the skewness of the distribution. Categorical variables are presented as n (%) and were compared with the chi-square test or, where expected counts were below five, with Fisher's exact test; comparisons in which Fisher's test was used are marked in Table 1.

The Mann-Whitney U test examines the hypothesis of equality of distributions against the alternative of stochastic dominance of one of them; the median and interquartile range serve a descriptive function in relation to it. Pairwise comparisons were planned before analysis and covered the comparison of empagliflozin with dapagliflozin and of each of the molecules with the group not receiving an SGLT2 inhibitor; their results are given in Tables 1 and 2, whereas the primary comparison with respect to biomarker concentrations, that is the comparison of all patients receiving an SGLT2 inhibitor with those not receiving one, is presented in Table 3 in crude and adjusted form. No omnibus test and no correction for multiplicity were applied to the comparisons presented in Tables 1 and 2. The p values in Table 1 serve to characterize the groups and are descriptive, whereas the comparison of empagliflozin with dapagliflozin with respect to the panel markers constitutes a secondary comparison planned before analysis.

The hierarchy adopted in the study comprises two separate dimensions: the marker domain defines the endpoint, and the groups compared define the comparison. The primary endpoint, that is the endothelial domain (Section 2.6), was assessed in the primary comparison defined in Section 2.3. Secondary are therefore, in the endpoint dimension, the oxidative domain and the integrative marker, and, in the comparison dimension, the direct comparison of empagliflozin with dapagliflozin.

The association of exposure with biomarker concentration was assessed with a single multiple regression model, applied in identical form to all analytes. The dependent variable was the log-transformed biomarker concentration and the explanatory variable was SGLT2 inhibitor use, with adjustment for age, sex, eGFR, type 2 diabetes, chronic heart failure, left ventricular ejection fraction, left atrial dimension and coronary artery disease. The covariates were selected before analysis as factors influencing both the decision to start an SGLT2 inhibitor and the concentrations of the analytes studied; no variable selection procedure and no criterion based on the results of univariable analysis were used. The regression coefficient for exposure was converted to the original scale as $(e^{\beta} - 1) \times 100\%$ and is presented as a percentage difference in concentration with a 95% confidence interval. Expressed in this way, the value is a relative difference in geometric means, conditional on the covariates; with a log-normal distribution of concentrations the geometric mean in a group coincides with the median, so that the adjusted estimate and the medians given in Table 3 refer to the same concentration scale, although they are not the same quantity, because the medians describe the crude comparison and the adjusted difference describes the comparison after the covariates have been taken into account. Model assumptions were assessed on the basis of residual analysis and of the tolerance of the explanatory variables, which exceeded 0.46 in all models. For sE-selectin, the only analyte with a left-skewed distribution, the distribution of the model residuals on log-transformed values departed from normality; once this departure had been identified, the model was repeated on untransformed values and the result is presented in Section 3.2. This was the only departure from the uniform form of the model.

A step-down Holm correction across the family of the six panel markers was applied to the p values from the adjusted models, in accordance with the principle set out in Section 2.6. Both clinical reference markers remained outside this family, because they belong neither to the panel nor to any of the

domains and serve to assess the set of covariates rather than to test the study hypothesis. The correction applied to the p values only; confidence intervals were not widened for multiplicity of comparisons. The corrected p values are given in Table 3.

Two sensitivity analyses were planned for the primary result. The first was repetition of the model after exclusion of influential observations, defined by a Cook's distance exceeding $4/n$. The second was a model extended by the log-transformed interleukin 6 concentration; this analyte was not included in the main model because its concentration differed according to exposure and was associated with sVCAM-1 concentration, so that it acts as a potential mediator rather than as a confounder. Two alternative specifications of the main model, extended by the presence of an implanted device and by NYHA class, are additionally presented in order to assess the stability of the estimate with respect to the choice of covariates. The results of all these models, together with the unadjusted model, are presented in Table S3.

A post hoc analysis, not covered by the plan adopted before analysis, was additionally performed. NYHA class III occurred exclusively in patients receiving an SGLT2 inhibitor, so that in the model covering the whole cohort this variable was estimated in a region without common support and adjustment for it could not, in that respect, balance the groups. The main model was therefore repeated in the subgroup of patients with chronic heart failure in NYHA class II, in which both groups were represented; this subgroup comprised 168 patients, including 127 receiving an SGLT2 inhibitor and 41 not receiving one, and included neither the 28 patients in class III nor the 91 patients without chronic heart failure. Chronic heart failure was omitted from the covariates, because it is constant within this subgroup; the remaining seven variables were retained unchanged. The analysis was performed for all eight analytes and is presented in Table S4. It is a sensitivity analysis of the primary result and not a separate family of confirmatory comparisons, and the Holm correction was therefore not applied to it again; the p value for sVCAM-1 after such a correction is given in the footnote to Table S4.

As an exploratory analysis, the association of the concentrations of the six panel markers with four indicators of the severity of cardiac disease — left ventricular ejection fraction, left atrial dimension, NT-proBNP concentration and high-sensitivity troponin T concentration — was assessed in the subgroup of patients with chronic heart failure using Spearman's rank correlation coefficient; for interpretation, the significance threshold after the Holm correction for the smallest of the 24 p values obtained is given (Table S2).

The power of the comparison of empagliflozin with dapagliflozin was estimated before analysis: with group sizes of 98 and 70 patients, a two-sided significance level of 0.05 and 80% power, a difference of the order of 0.44 standard deviations was detectable for the independent-samples Student t test.

Measurements of the six panel markers were complete in all 287 participants, as were all covariates used in the models. Concentrations of NT-proBNP, high-sensitivity troponin T and procalcitonin were not obtained in the same two patients, both from the group not receiving an SGLT2 inhibitor, and comparisons involving these analytes were therefore based on 168 treated and 117 untreated patients.

In one of these two patients, concentrations of interleukin 6, albumin and bilirubin and the activity of both aminotransferases were additionally not obtained; comparisons involving these variables were based on 168 treated and 118 untreated patients, and the model extended by interleukin 6 on 286 patients (Table S3). No imputation was used; no other variable contained missing values.

2.8. Ethics approval

The study was conducted in accordance with the principles of the Declaration of Helsinki. The protocol was approved by the Bioethics Committee at Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (no. KB 295/2024). All participants gave voluntary, informed, written consent before enrollment in the study.

3. Results

3.1. Cohort characteristics

The analysis included 287 patients, among them 205 men (71.4%), with a mean age of 65.21 ± 9.46 years and a mean left ventricular ejection fraction of $47.77 \pm 14.62\%$. Chronic heart failure was present in 196 patients (68.3%), type 2 diabetes in 88 (30.7%) and chronic kidney disease of at least stage 3 in 58 (20.2%). A direct oral anticoagulant was received by 266 patients (92.7%). An SGLT2 inhibitor was taken by 168 patients (58.5%): empagliflozin by 98 and dapagliflozin by 70. The full characteristics, comprising 48 clinical and laboratory variables, are presented in Table 1, and the distribution of patients according to exposure in Fig. 1.

Table 1
Clinical and laboratory characteristics of the patients according to the SGLT2 inhibitor used

Parameter	Empagliflozin (n = 98)	Dapagliflozin (n = 70)	No SGLT2i (n = 119)	p (1:2)	p (1:0)	p (2:0)
Age (years)☐	64.8 ± 8.4	64.9 ± 9.0	65.7 ± 10.5	0.927	0.463	0.579
Male sex, n (%)☐	80 (81.6)	55 (78.6)	70 (58.8)	0.622	< 0.001	0.006
BMI (kg/m ²)	30.1 ± 5.6	29.1 ± 6.2	30.0 ± 6.3	0.237	0.845	0.329
CHA ₂ DS ₂ -VA	3.03 ± 1.45	3.06 ± 1.58	2.35 ± 1.27	0.911	< 0.001	< 0.001
HAS-BLED	0.79 ± 0.74	0.86 ± 0.84	0.74 ± 0.66	0.559	0.626	0.286
Atrial fibrillation type: paroxysmal / persistent / permanent, n (%)	44 (44.9) / 37 (37.8) / 17 (17.3)	33 (47.1) / 20 (28.6) / 17 (24.3)	76 (63.9) / 35 (29.4) / 8 (6.7)	0.362☐	0.007☐	0.002☐
Sinus rhythm at the time of sampling, n (%)	66 (67.3)	48 (68.6)	92 (77.3)	0.867	0.101	0.186
Chronic heart failure, n (%)☐	89 (90.8)	66 (94.3)	41 (34.5)	0.407	< 0.001	< 0.001
NYHA class: II / III, n (%)☐	73 (82.0) / 16 (18.0)	54 (81.8) / 12 (18.2)	41 (100) / 0 (0)	0.974☐	0.004☐	0.003☐☐
Ischemic cardiomyopathy, n (%)	29 (29.6)	21 (30.0)	8 (6.7)	0.955	< 0.001	< 0.001
Implanted device, n (%)	29 (29.6)	33 (47.1)	11 (9.2)	0.020	< 0.001	< 0.001
Arterial hypertension, n (%)	75 (76.5)	55 (78.6)	90 (75.6)	0.755	0.877	0.644
Type 2 diabetes, n (%)☐	36 (36.7)	25 (35.7)	27 (22.7)	0.892	0.023	0.053
Dyslipidemia, n (%)	87 (88.8)	62 (88.6)	91 (76.5)	0.967	0.019	0.041
Coronary artery disease, n (%)☐	36 (36.7)	26 (37.1)	20 (16.8)	0.957	< 0.001	0.002
CKD stage ≥ 3, n (%)	25 (25.5)	21 (30.0)	12 (10.1)	0.520	0.003	< 0.001

Parameter	Empagliflozin (n = 98)	Dapagliflozin (n = 70)	No SGLT2i (n = 119)	p (1:2)	p (1:0)	p (2:0)
Direct oral anticoagulant, n (%)	90 (91.8)	63 (90.0)	113 (95.0)	0.681	0.352	0.237
Beta-blocker, n (%)	95 (96.9)	68 (97.1)	108 (90.8)	1.000	0.065	0.137
ARNI, n (%)	46 (46.9)	35 (50.0)	5 (4.2)	0.695	< 0.001	< 0.001
Angiotensin-converting enzyme inhibitor, n (%)	38 (38.8)	19 (27.1)	49 (41.2)	0.116	0.719	0.052
Angiotensin receptor blocker, n (%)	6 (6.1)	13 (18.6)	34 (28.6)	0.012	< 0.001	0.125
Renin-angiotensin system blockade (angiotensin-converting enzyme inhibitor, angiotensin receptor blocker or ARNI), n (%)	90 (91.8)	67 (95.7)	87 (73.1)	0.364	< 0.001	< 0.001
Mineralocorticoid receptor antagonist, n (%)	67 (68.4)	54 (77.1)	23 (19.3)	0.212	< 0.001	< 0.001
Digoxin, n (%)	12 (12.2)	8 (11.4)	8 (6.7)	0.872	0.162	0.262
Antiarrhythmic drug, n (%)	25 (25.5)	17 (24.3)	46 (38.7)	0.857	0.040	0.043
Statin, n (%)	83 (84.7)	62 (88.6)	81 (68.1)	0.471	0.005	0.002
Ezetimibe, n (%)	36 (36.7)	29 (41.4)	23 (19.3)	0.538	0.004	0.001
LVEF (%)	41.5 ± 13.9	39.1 ± 13.4	58.0 ± 8.5	0.271	< 0.001	< 0.001
LVDd (mm)	57.5 ± 9.2	58.4 ± 8.6	49.2 ± 5.9	0.561	< 0.001	< 0.001
LVDs (mm)	45.4 ± 11.8	45.8 ± 11.4	34.6 ± 6.2	0.790	< 0.001	< 0.001
Left atrial dimension (mm)	50.1 ± 7.1	50.2 ± 6.0	45.4 ± 6.2	0.911	< 0.001	< 0.001
Sodium (mmol/l)	140.6 ± 2.5	140.2 ± 2.3	140.1 ± 2.3	0.265	0.131	0.838

Parameter	Empagliflozin (n = 98)	Dapagliflozin (n = 70)	No SGLT2i (n = 119)	p (1:2)	p (1:0)	p (2:0)
Potassium (mmol/l)	4.55 ± 0.38	4.55 ± 0.39	4.34 ± 0.32	0.968	< 0.001	< 0.001
Creatinine (mg/dl)	1.09 [0.93–1.30]	1.08 [0.93–1.38]	0.96 [0.83–1.12]	0.875	< 0.001	< 0.001
eGFR (ml/min/1.73 m ²) [§]	68.1 ± 19.5	67.0 ± 22.3	73.2 ± 18.3	0.722	0.052	0.041
Glucose (mg/dl)	107 [97–124]	110 [96–134]	100 [92–115]	0.539	0.010	0.011
AST (U/l)	23.0 [20.0–28.0]	22.5 [19.0–27.0]	24.0 [18.2–29.0]	0.394	0.560	0.871
ALT (U/l)	23.0 [17.0–30.0]	23.0 [18.2–30.8]	22.0 [17.0–32.0]	0.842	0.976	0.806
Bilirubin (mg/dl)	0.63 [0.46–0.83]	0.56 [0.44–0.81]	0.60 [0.47–0.84]	0.438	0.677	0.197
hsCRP (mg/l)	1.74 [0.85–3.50]	1.58 [0.98–3.99]	1.19 [0.77–2.92]	0.691	0.102	0.174
Interleukin 6 (pg/ml)	5.30 [3.73–7.55]	5.00 [3.10–8.07]	3.90 [2.62–6.30]	0.968	0.003	0.008
Procalcitonin (ng/ml)	0.050 [0.040–0.080]	0.050 [0.040–0.070]	0.040 [0.030–0.060]	0.409	< 0.001	0.023
Albumin (g/dl)	4.47 [4.23–4.65]	4.44 [4.28–4.59]	4.48 [4.27–4.62]	0.456	0.761	0.326
Leukocytes (G/l)	7.32 [6.09–8.29]	6.94 [6.25–7.99]	6.35 [5.31–7.21]	0.425	< 0.001	0.008
Hemoglobin (g/dl)	15.2 ± 1.6	15.0 ± 1.5	14.2 ± 1.5	0.438	< 0.001	< 0.001
RDW (%)	13.5 [13.0–14.6]	13.8 [13.2–14.6]	13.2 [12.7–13.9]	0.454	0.009	0.002

Parameter	Empagliflozin (n = 98)	Dapagliflozin (n = 70)	No SGLT2i (n = 119)	p (1:2)	p (1:0)	p (2:0)
Platelets (G/l)	208 ± 48	213 ± 57	211 ± 53	0.533	0.610	0.855
TSH (μIU/ml)	2.02 [1.46– 3.50]	1.84 [1.28– 3.09]	1.74 [1.17– 2.56]	0.287	0.011	0.168

Values are presented as mean ± standard deviation, median [interquartile range] or n (%). The form of description indicates the test used: mean ± standard deviation – independent-samples Student t test; median [interquartile range] – Mann-Whitney U test; n (%) – chi-square test, or Fisher's exact test where expected counts were below five. Groups: 1 – empagliflozin, 2 – dapagliflozin, 0 – no SGLT2 inhibitor. The p values come from pairwise comparisons and were not corrected for multiplicity. ¶ The p value concerns the distribution of the three types of atrial fibrillation taken together (chi-square test in a 2 × 3 layout) and not the individual types. ¶ Fisher's exact test; in these comparisons at least one expected count was smaller than five. ¶ Variable included as a covariate in the multiple regression model whose results are presented in Table 3 and Fig. 2. ¶ NYHA class was assessed only in patients with chronic heart failure; the percentages refer to the number of such patients in each of the groups (89, 66 and 41, respectively). No patient was in class I or IV, and the p value therefore concerns the distribution of classes II and III. ARNI – angiotensin receptor-neprilysin inhibitor; BMI – body mass index; eGFR – estimated glomerular filtration rate; hsCRP – high-sensitivity C-reactive protein; LVDd – left ventricular end-diastolic dimension; LVDs – left ventricular end-systolic dimension; LVEF – left ventricular ejection fraction; CKD – chronic kidney disease; RDW – red cell distribution width; TSH – thyrotropin.

Empagliflozin and dapagliflozin differed from one another in two of the 48 variables compared: the frequency of an implanted device (29.6% versus 47.1%; p = 0.020) and the use of an angiotensin receptor blocker (6.1% versus 18.6%; p = 0.012). No difference was found for any of the remaining variables (all p ≥ 0.116). The number of significant results corresponds to the number expected with 48 comparisons conducted at a significance level of 0.05, and these comparisons were not corrected for multiplicity.

Patients receiving either SGLT2 inhibitor did, by contrast, differ from those not receiving one in many components of the clinical profile, with the direction of the differences concordant for both molecules. The values below are given for empagliflozin and dapagliflozin versus the group not receiving an SGLT2 inhibitor.

Treated patients were more often men (81.6% and 78.6% versus 58.8%; p < 0.001 and p = 0.006) and had a higher CHA₂DS₂-VA score (3.03 ± 1.45 and 3.06 ± 1.58 versus 2.35 ± 1.27; both p < 0.001). Age, body mass index and HAS-BLED score did not differentiate the groups (all p ≥ 0.286). The distribution of the types of atrial fibrillation differed (p = 0.007 and p = 0.002): paroxysmal atrial fibrillation was diagnosed in 44.9% and 47.1% versus 63.9%, persistent in 37.8% and 28.6% versus 29.4%, and permanent in 17.3%

and 24.3% versus 6.7%. The proportion of patients in sinus rhythm at the time of blood sampling was similar (67.3% and 68.6% versus 77.3%; $p = 0.101$ and $p = 0.186$).

More frequent were: chronic heart failure (90.8% and 94.3% versus 34.5%), ischemic cardiomyopathy (29.6% and 30.0% versus 6.7%) and an implanted device (29.6% and 47.1% versus 9.2%) – all $p < 0.001$ – as well as coronary artery disease (36.7% and 37.1% versus 16.8%; $p < 0.001$ and $p = 0.002$), chronic kidney disease of at least stage 3 (25.5% and 30.0% versus 10.1%; $p = 0.003$ and $p < 0.001$) and dyslipidemia (88.8% and 88.6% versus 76.5%; $p = 0.019$ and $p = 0.041$). Type 2 diabetes was more frequent in patients receiving empagliflozin (36.7% versus 22.7%; $p = 0.023$), whereas for dapagliflozin the difference did not reach the significance level adopted (35.7%; $p = 0.053$). Among the 196 patients with chronic heart failure, NYHA class III occurred exclusively in those receiving an SGLT2 inhibitor: in 16 of 89 patients treated with empagliflozin (18.0%) and in 12 of 66 treated with dapagliflozin (18.2%) versus none of the 41 not receiving an SGLT2 inhibitor ($p = 0.004$ and $p = 0.003$); the remaining patients were in class II, and no patient was in class I or IV; the two molecules did not differ from one another in this respect ($p = 0.974$). The frequency of arterial hypertension was similar in all three groups ($p = 0.877$ and $p = 0.644$).

On echocardiography, treated patients had a lower left ventricular ejection fraction ($41.5 \pm 13.9\%$ and $39.1 \pm 13.4\%$ versus $58.0 \pm 8.5\%$), a larger left ventricular end-diastolic dimension (57.5 ± 9.2 and 58.4 ± 8.6 versus 49.2 ± 5.9 mm) and end-systolic dimension (45.4 ± 11.8 and 45.8 ± 11.4 versus 34.6 ± 6.2 mm), and a larger left atrial dimension (50.1 ± 7.1 and 50.2 ± 6.0 versus 45.4 ± 6.2 mm); all $p < 0.001$.

Concomitant therapy was more intensive: an ARNI (46.9% and 50.0% versus 4.2%) and a mineralocorticoid receptor antagonist (68.4% and 77.1% versus 19.3%) – both $p < 0.001$ – as well as a statin (84.7% and 88.6% versus 68.1%; $p = 0.005$ and $p = 0.002$) and ezetimibe (36.7% and 41.4% versus 19.3%; $p = 0.004$ and $p = 0.001$) were used more frequently, whereas an antiarrhythmic drug was used less frequently (25.5% and 24.3% versus 38.7%; $p = 0.040$ and $p = 0.043$). An angiotensin receptor blocker was received by 6.1% of patients treated with empagliflozin versus 28.6% of those not receiving an SGLT2 inhibitor ($p < 0.001$), with no difference for dapagliflozin (18.6%; $p = 0.125$). Renin-angiotensin system blockade, understood as the use of an angiotensin-converting enzyme inhibitor, an angiotensin receptor blocker or an ARNI, was received by 91.8% of patients treated with empagliflozin and 95.7% of those treated with dapagliflozin versus 73.1% of those not receiving an SGLT2 inhibitor (both $p < 0.001$). The frequency of use of a direct oral anticoagulant, a beta-blocker, an angiotensin-converting enzyme inhibitor and digoxin did not differ between the groups (all $p \geq 0.052$; Fisher's exact test was used for the beta-blocker and the direct oral anticoagulant).

On laboratory testing, treated patients had higher concentrations of potassium (4.55 ± 0.38 and 4.55 ± 0.39 versus 4.34 ± 0.32 mmol/l) and creatinine ($1.09 [0.93-1.30]$ and $1.08 [0.93-1.38]$ versus $0.96 [0.83-1.12]$ mg/dl; all $p < 0.001$), with a lower eGFR, the difference in which reached the significance level adopted for dapagliflozin (67.0 ± 22.3 versus 73.2 ± 18.3 ml/min/1.73 m²; $p = 0.041$) but did not reach it for empagliflozin (68.1 ± 19.5 ; $p = 0.052$). Also higher were the glucose concentration ($107 [97-124]$ and

110 [96–134] versus 100 [92–115] mg/dl; $p = 0.010$ and $p = 0.011$), the hemoglobin concentration (15.2 ± 1.6 and 15.0 ± 1.5 versus 14.2 ± 1.5 g/dl; both $p < 0.001$), the leukocyte count (7.32 [6.09–8.29] and 6.94 [6.25–7.99] versus 6.35 [5.31–7.21] G/l; $p < 0.001$ and $p = 0.008$) and RDW (13.5 [13.0–14.6] and 13.8 [13.2–14.6] versus 13.2 [12.7–13.9]%; $p = 0.009$ and $p = 0.002$).

Inflammatory indices behaved divergently: the concentrations of interleukin 6 (5.30 [3.73–7.55] and 5.00 [3.10–8.07] versus 3.90 [2.62–6.30] pg/ml; $p = 0.003$ and $p = 0.008$) and of procalcitonin (0.050 [0.040–0.080] and 0.050 [0.040–0.070] versus 0.040 [0.030–0.060] ng/ml; $p < 0.001$ and $p = 0.023$) were higher in treated patients, whereas the high-sensitivity C-reactive protein concentration did not differentiate the groups (1.74 [0.85–3.50] and 1.58 [0.98–3.99] versus 1.19 [0.77–2.92] mg/l; $p = 0.102$ and $p = 0.174$). The TSH concentration was higher in patients receiving empagliflozin (2.02 [1.46–3.50] versus 1.74 [1.17–2.56] μ IU/ml; $p = 0.011$), with no difference for dapagliflozin (1.84 [1.28–3.09]; $p = 0.168$). Sodium concentration, aminotransferase activity, bilirubin and albumin concentrations and platelet count did not differentiate the groups (all $p \geq 0.131$).

3.2. The biomarker panel and SGLT2 inhibitor use

Biomarker concentrations in the three groups are presented in Table 2. Empagliflozin and dapagliflozin did not differ in any of the six panel markers or in either of the clinical reference markers; the smallest p value was 0.156 and concerned sVCAM-1. With group sizes of 98 and 70 patients and 80% power, a standardized difference of the order of 0.44 standard deviations was detectable, so that this comparison excludes moderate and large differences but does not exclude small ones.

Table 2
Biomarker concentrations according to the molecule used

Biomarker	Empagliflozin (n = 98)	Dapagliflozin (n = 70)	No SGLT2i (n = 119)	p (1:2)	p (1:0)	p (2:0)
sVCAM-1 (ng/ml)	172.2 [157.9– 262.0]	178.1 [160.7– 261.8]	157.9 [122.0– 161.7]	0.156	< 0.001	< 0.001
sE-selectin (ng/ml)	107.9 [97.7– 116.8]	109.7 [92.8– 118.3]	101.4 [88.6– 110.6]	0.963	0.001	0.006
Angiopoietin-2 (pg/ml)	2388 [1687– 3524]	2536 [1522– 3418]	2862 [1934– 4418]	0.720	0.011	0.061
8-OHdG (pg/ml)	400.0 [292.9– 547.5]	403.4 [319.7– 521.9]	402.0 [289.0– 601.3]	0.796	0.567	0.868
AOPP (µg/ml)	133.9 [80.9– 191.4]	124.1 [84.7– 186.2]	131.3 [76.4– 193.1]	0.999	0.926	0.942
GDF-15 (pg/ml)	1623 [480– 16,156]	1300 [274– 9062]	3048 [826– 19,001]	0.217	0.140	0.008
NT-proBNP (pg/ml)⊠	664 [268–1260]	674 [248–1352]	373 [141– 804]	0.688	0.003	0.002
hs-TnT (ng/l)⊠	14.0 [10.0–21.0]	16.0 [11.0– 25.8]	11.0 [8.0– 17.0]	0.414	< 0.001	< 0.001

Values are given as median [interquartile range]; comparisons with the Mann-Whitney U test. The analytes are ordered according to the domains assigned before analysis: endothelial (sVCAM-1, sE-selectin, angiopoietin-2), oxidative (8-OHdG, AOPP) and integrative (GDF-15). Groups: 1 – empagliflozin, 2 – dapagliflozin, 0 – no SGLT2 inhibitor. The p values were not corrected for multiplicity; the Holm correction was applied only to the adjusted models (Table 3). Empagliflozin and dapagliflozin did not differ in any of the eight analytes; the smallest p value was 0.156. ⊠ NT-proBNP and troponin T do not belong to the panel; they are presented as clinical reference markers, and the group without an SGLT2 inhibitor comprises 117 patients for them. Concentrations of advanced oxidation protein products are given in µg/ml; the kit reports the result in ng/ml (1 µg/ml = 1000 ng/ml; Table S1). AOPP – advanced oxidation protein products; GDF-15 – growth differentiation factor 15; hs-TnT – high-sensitivity troponin T; NT-proBNP – N-terminal pro-B-type natriuretic peptide; 8-OHdG – 8-hydroxy-2'-deoxyguanosine; sE-selectin – soluble E-selectin (CD62E); sVCAM-1 – soluble vascular cell adhesion molecule 1 (CD106).

The comparison of each molecule separately with the group not receiving an SGLT2 inhibitor was not uniform across the whole panel. Both molecules differed from the untreated group with respect to sVCAM-1 (both p < 0.001) and sE-selectin (p = 0.001 and p = 0.006). For angiopoietin-2 the difference

reached the significance level adopted only for empagliflozin (2388 versus 2862 pg/ml; $p = 0.011$; dapagliflozin 2536 pg/ml, $p = 0.061$), and for GDF-15 only for dapagliflozin (1300 versus 3048 pg/ml; $p = 0.008$; empagliflozin 1623 pg/ml, $p = 0.140$). The direct comparison of the two molecules showed no difference for either of these two analytes ($p = 0.720$ and $p = 0.217$), and both lost their association with exposure after adjustment for clinical variables (Table 3). This divergence therefore reflects the resolution of comparisons conducted in smaller subgroups rather than different behavior of the molecules. Neither analyte of the oxidative domain differentiated either molecule from the untreated group (8-hydroxy-2'-deoxyguanosine $p = 0.567$ and $p = 0.868$; advanced oxidation protein products $p = 0.926$ and $p = 0.942$), whereas both clinical reference markers differentiated both molecules from it (NT-proBNP $p = 0.003$ and $p = 0.002$; troponin T both $p < 0.001$).

In the crude comparison, patients receiving an SGLT2 inhibitor had higher concentrations of sVCAM-1 (median 177.1 versus 157.9 ng/ml) and sE-selectin (108.9 versus 101.4 ng/ml; both p values < 0.001) and lower concentrations of angiopoietin-2 (2454 versus 2862 pg/ml; $p = 0.008$) and GDF-15 (1445 versus 3048 pg/ml; $p = 0.017$). Neither analyte of the oxidative domain differentiated the groups: 8-hydroxy-2'-deoxyguanosine 402.9 versus 402.0 pg/ml ($p = 0.639$) and advanced oxidation protein products 132.6 versus 131.3 μ g/ml ($p = 0.920$). The clinical reference markers differentiated the groups in line with the difference in clinical profile described above: NT-proBNP 664 versus 373 pg/ml and troponin T 15.0 versus 11.0 ng/l (both p values < 0.001). The separation of the two domains was therefore already apparent in the crude comparison: all three endothelial analytes differentiated the groups, and neither of the two oxidative analytes did so.

After adjustment for age, sex, eGFR, type 2 diabetes, chronic heart failure, ejection fraction, left atrial dimension and coronary artery disease, only sVCAM-1 retained an association with exposure: the concentration was 12.8% higher (95% CI + 3.6 to + 22.9; $p = 0.006$). The differences for sE-selectin, angiopoietin-2 and GDF-15 disappeared ($p = 0.178$, 0.930 and 0.951, respectively), and both analytes of the oxidative domain remained non-differentiating ($p = 0.657$ and 0.764). The same set of variables also abolished entirely the differences in the clinical reference markers: NT-proBNP – 12.7% ($p = 0.427$) and troponin T – 1.8% ($p = 0.831$). After the Holm correction across the six panel markers, only the result for sVCAM-1 remained significant (corrected p 0.036), with a threshold for the smallest uncorrected p value of 0.0083; for the remaining panel analytes the corrected p values ranged from 0.890 to 1.000. For sE-selectin, in which the distribution of the model residuals on log-transformed values departed from normality, the model was repeated on untransformed values; the result remained non-significant (+ 5.78 ng/ml; 95% CI – 2.10 to + 13.67; $p = 0.150$), and the uniform specification was therefore retained for the whole panel. The data are presented in Table 3 and Fig. 2.

Table 3
Biomarker concentrations and adjusted differences by SGLT2 inhibitor exposure

Biomarker	SGLT2i (n = 168)	No SGLT2i (n = 119)	Crude p	Adjusted difference (%)	95% CI	Adjusted p	Holm- adjusted p
sVCAM-1 (ng/ml)	177.1 [158.6– 262.2]	157.9 [122.0– 161.7]	< 0.001	+ 12.8	+ 3.6 to + 22.9	0.006	0.036
sE-selectin (ng/ml)	108.9 [95.2– 117.5]	101.4 [88.6– 110.6]	< 0.001	+ 11.1	–4.7 to + 29.6	0.178	0.890
Angiopoietin- 2 (pg/ml)	2454 [1635– 3496]	2862 [1934– 4418]	0.008	–1.0	–20.8 to + 23.8	0.930	1.000
8-OHdG (pg/ml)	402.9 [309.1– 533.6]	402.0 [289.0– 601.3]	0.639	–4.2	–20.8 to + 15.8	0.657	1.000
AOPP (µg/ml)	132.6 [83.2– 188.9]	131.3 [76.4– 193.1]	0.920	+ 3.5	–17.4 to + 29.7	0.764	1.000
GDF-15 (pg/ml)	1445 [355– 12,135]	3048 [826– 19,001]	0.017	–2.0	–48.9 to + 87.8	0.951	1.000
NT-proBNP (pg/ml)⊠	664 [256– 1286]	373 [141– 804]	< 0.001	–12.7	–37.7 to + 22.3	0.427	not applicable (outside panel)
hs-TnT (ng/l)⊠	15.0 [10.0– 23.0]	11.0 [8.0– 17.0]	< 0.001	–1.8	–17.3 to + 16.5	0.831	not applicable (outside panel)

Values are medians [interquartile ranges]; crude p values are from the Mann-Whitney U test. Adjusted differences were estimated by regression of log-transformed concentrations controlling for age, sex, eGFR, type 2 diabetes, chronic heart failure, left ventricular ejection fraction, left atrial dimension and coronary artery disease. Coefficients were transformed as $(e^{\beta} - 1) \times 100\%$; positive values indicate higher geometric means with SGLT2 inhibitor use. Holm-adjusted p values cover the six panel markers; confidence intervals were unadjusted. NT-proBNP and hs-TnT were reference markers outside this family and were analyzed in 285 participants. Because sE-selectin residuals were non-normal, an untransformed sensitivity model gave + 5.78 ng/ml (95% CI –2.10 to + 13.67; p = 0.150). Abbreviations are defined in Table 2.

The forest plot shows the adjusted relative difference in geometric mean concentration for each analyte with its 95% confidence interval. Positive values indicate higher concentrations with SGLT2 inhibitor use

and negative values indicate lower concentrations; the dashed vertical line indicates no difference. The logarithmic axis places reciprocal concentration changes at equal distances from the reference line. Exact estimates and p values are reported in Table 3.

Each multiple regression model used the log-transformed analyte concentration as the dependent variable and adjusted for age, sex, eGFR, type 2 diabetes, chronic heart failure, left ventricular ejection fraction, left atrial dimension and coronary artery disease. The exposure coefficient was transformed as $(e^{\beta} - 1) \times 100\%$. Analytes were grouped a priori as endothelial (sVCAM-1, sE-selectin and angiopoietin-2), oxidative (8-OHdG and AOPP) or integrative (GDF-15). NT-proBNP and hs-TnT were clinical reference markers outside the biomarker panel.

A filled marker identifies sVCAM-1, the only panel analyte significant after multivariable adjustment ($p = 0.006$) and Holm correction across six panel markers (adjusted $p = 0.036$). Confidence intervals were not adjusted for multiplicity. Estimates used 287 participants, except those for NT-proBNP and hs-TnT, which used 285 because values were unavailable for two untreated patients. The cross-sectional estimates represent associations, not treatment effects or changes over time. Abbreviations: AOPP, advanced oxidation protein products; CI, confidence interval; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor 15; hs-TnT, high-sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SGLT2, sodium-glucose co-transporter 2; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

3.3. Sensitivity analyses

The result for sVCAM-1 was subjected to sensitivity analyses; details are presented in Table S3. In the unadjusted model the concentration was 25.9% higher (95% CI + 18.1 to + 34.2; $p < 0.001$).

After exclusion of nine influential observations, defined by a Cook's distance exceeding $4/n$, the association remained unchanged (+ 12.9%; 95% CI + 4.1 to + 22.4; $p = 0.004$).

After additional adjustment for the log-transformed interleukin 6 concentration, the association did not weaken (+ 13.7%; 95% CI + 4.5 to + 23.7; $p = 0.003$), which indicates that it is not mediated by systemic inflammatory activation. Interleukin 6 was not included in the main model because of its role as a potential mediator: its concentration differed according to exposure (Table 1) and was associated with sVCAM-1 concentration.

The estimate was stable with respect to the choice of covariates. After extension of the main model by the presence of an implanted device it was + 14.1% (95% CI + 4.8 to + 24.2; $p = 0.003$), and after extension by NYHA class + 12.8% (95% CI + 3.5 to + 23.0; $p = 0.006$).

The main model was then repeated in the subgroup of 168 patients in NYHA class II (127 receiving an SGLT2 inhibitor and 41 not receiving one), after exclusion of 28 patients in class III, all of whom belonged to the treated group, and of 91 patients without chronic heart failure. The association did not weaken: the sVCAM-1 concentration was 15.7% higher (95% CI + 2.5 to + 30.7; $p = 0.018$); the medians were 178.0 versus 160.3 ng/ml. This subgroup remained unbalanced with respect to ejection fraction (40.2 ± 12.8

versus $52.9 \pm 11.1\%$) and age (64.2 ± 8.6 versus 68.6 ± 9.5 years), which continued to be adjusted for in the model; the restriction removed only that component of the imbalance for which adjustment by modeling was not possible.

The behavior of the clinical reference markers in this subgroup was the same as in the model covering the whole cohort. Without adjustment, the NT-proBNP concentration was 80.1% higher in treated patients (95% CI + 34.0 to + 142.0) and the troponin T concentration 34.8% higher (95% CI + 16.7 to + 55.8); both differences were abolished by adjustment for clinical variables (-12.7% , $p = 0.427$ and -1.8% , $p = 0.831$) and by restriction of the analysis to NYHA class II (-22.7% , $p = 0.213$ and -3.8% , $p = 0.705$) alike. None of the remaining five panel markers reached the significance level adopted in this subgroup (p from 0.346 to 0.915). The comparison of all eight analytes in the unadjusted model, in the main model and in the restricted analysis is presented in Table S4.

The proportion of variance explained ranged across all specifications from 10.2% to 27.0% (Table S3); the lowest value was obtained in the analysis restricted to NYHA class II, which corresponds to the narrower range of variability of the covariates in this subgroup. Sensitivity analyses with respect to model specification were performed for the primary result, that is for sVCAM-1 – the only analyte whose association with exposure reached significance in the main model; the analysis restricted to NYHA class II was performed for all eight analytes.

3.4. Association of the panel with indicators of the severity of cardiac disease

In the subgroup of 196 patients with chronic heart failure, all six panel measurements were compared with four indicators of the severity of cardiac disease. An association with the echocardiographic indicators was shown only by the two adhesion molecules: sVCAM-1 with left ventricular ejection fraction ($p = -0.25$; $p = 0.0004$) and with left atrial dimension ($p = +0.19$; $p = 0.008$), and sE-selectin with left atrial dimension ($p = +0.18$; $p = 0.014$). Angiopoietin-2, GDF-15, 8-hydroxy-2'-deoxyguanosine and advanced oxidation protein products showed no association with any of the four indicators (p from -0.12 to $+0.10$; all $p \geq 0.085$). None of the six analytes was associated with NT-proBNP or high-sensitivity troponin T concentration. After the Holm correction across 24 comparisons, with a threshold for the smallest p value of 0.0021, only the association of sVCAM-1 with ejection fraction remained significant. The data are presented in Table S2.

4. Discussion

4.1. Main results

Three observations emerge from this cross-sectional analysis. Markers of endothelial activation distinguished patients receiving an SGLT2 inhibitor from those not receiving one, whereas markers of oxidative damage to DNA and to proteins did not distinguish them in any analysis. Of the whole panel, only sVCAM-1 retained an independent association with exposure after adjustment for clinical variables.

Empagliflozin and dapagliflozin, by contrast, were statistically indistinguishable across all eight analytes measured.

4.2. Dissociation of the endothelial and oxidative domains

The divergence of the two domains is the most informative element of these data. Biomarker panels are usually presented as unordered lists, which obscures the possibility that individual domains behave differently. Here they behaved differently in a clear-cut way: the endothelial analytes shifted in a consistent direction, and neither oxidative analyte shifted at all. Several explanations are consistent with this. Circulating markers of oxidative damage reflect the systemic redox balance on a time scale that need not correspond to the local vascular processes reported by adhesion molecules; their concentration depends, moreover, not only on the generation of damage but also on its repair and elimination. This divergence also has a counterpart in the very construction of the preclinical evidence: the antioxidant premise for this class of drugs was built on measurements of reactive oxygen species in the cytoplasm and mitochondria of animal cardiomyocytes and in cell cultures, and not on circulating end products of the oxidation of DNA and proteins [11]. The lipid component of the oxidative domain was not measured in this analysis, so that this domain is represented by two analytes corresponding to two of the three classes of macromolecules susceptible to oxidation. The absence of a shift does not, moreover, prove that these analytes respond to nothing: in a systematic review with meta-analysis covering patients with heart failure, 8-hydroxy-2'-deoxyguanosine concentrations were higher than in healthy controls in all the studies included [29]. The axis of comparison there was, however, different from that in the present work — disease versus health, rather than exposure versus its absence — and advanced oxidation protein products were not covered by that analysis.

The divergence of the two domains is apparent as early as the level of the crude comparisons, before any adjustment: all three endothelial analytes differentiated patients receiving and not receiving an SGLT2 inhibitor, whereas neither of the two oxidative analytes differentiated them, the medians being practically identical (402.9 versus 402.0 pg/ml for 8-hydroxy-2'-deoxyguanosine). After adjustment for clinical variables the picture became simpler still: only sVCAM-1 retained an association, whereas the differences for sE-selectin, angiopoietin-2 and GDF-15 disappeared — just as did the differences in NT-proBNP and troponin T.

4.3. Measurement validity within the panel

A biomarker panel in which no analyte shows an association with known indicators of disease severity is difficult to interpret, because it does not allow the absence of a difference to be distinguished from the absence of measurement sensitivity. Comparing all six measurements with indicators of the severity of cardiac disease within a clinically defined stratum therefore answers a question that precedes all the others: whether these measurements register clinically meaningful variability in this cohort at all.

The distribution of the results was not uniform and followed the division into domains adopted before analysis. An association with the echocardiographic indicators was shown only by the two adhesion molecules: sVCAM-1 with ejection fraction and with left atrial dimension, and sE-selectin with left atrial

dimension. The directions were as expected — a lower ejection fraction and a larger atrium corresponded to higher concentrations. After the Holm correction, only the association of sVCAM-1 with ejection fraction met the significance criterion among them. Angiopoietin-2, GDF-15 and both analytes of the oxidative domain showed no association with any of the four indicators. This observation matters for the reading of the negative result in the oxidative domain, although in a more cautious direction than might appear. The absence of differences according to exposure cannot be attributed to sample size alone, given that the same measurements also fail to register the severity of cardiac disease, which varies over a wide range in this cohort. The test set out at the beginning of this section is therefore met differently for the two domains: for the adhesion molecules it is met, for the oxidative analytes it is not, so that their null result remains ambiguous between a true absence of a difference and limited sensitivity of these measurements in the material studied. The distinctness of angiopoietin-2 from the two adhesion molecules corresponds, moreover, to a mechanistic difference: angiopoietin-2 is not a leukocyte adhesion molecule and, when Tie2 signaling is attenuated, may destabilize endothelial junctions through activation of the $\beta 1$ integrin [17], so that its assignment to the endothelial domain need not mean that it behaves like the adhesion molecules. The distribution of the imaging associations also has an external counterpart. In a subgroup of 933 individuals without diagnosed atrial fibrillation or heart failure who underwent cardiac magnetic resonance imaging, a higher sVCAM-1 concentration corresponded to a lower left atrial emptying fraction ($\beta - 2.51$; 95% CI $- 4.33$ to $- 0.70$). No association was found, by contrast, with indexed left atrial volume, with left ventricular ejection fraction, with its end-systolic volume or with circumferential strain [22]. In a population with a narrow range of variability of left ventricular function, sVCAM-1 was therefore associated with an atrial rather than a ventricular measure; in our cohort, in which the ejection fraction ranged from 15% to 72%, the association is apparent with respect to that indicator as well.

This observation should nevertheless be read with a reservation. These data indicate that the variability of the adhesion molecules in this cohort lies along the same axis as the severity of disease, whereas the resolution of a cross-sectional design does not allow it to be determined which of these two quantities is primary in these data. Establishing this would require a longitudinal design with repeated measurement of both quantities.

4.4. Comparison of empagliflozin and dapagliflozin

The absence of a difference between empagliflozin and dapagliflozin deserves to be presented as a result in its own right, because it bears directly on the clinical decision. The two molecules have not been compared directly in a randomized trial, and indirect juxtaposition of the results of separate studies does not allow the molecule to be separated from the population in which it was assessed. In a single cohort, with measurements performed on the same plates and by the same operator for a given analyte, the two molecules yielded biomarker profiles that were statistically indistinguishable across all eight analytes measured, the smallest p value being 0.156.

The direct comparisons available are few and do not form a uniform picture. The only prospective, within-patient comparison to date covered 25 patients with stable chronic heart failure, in whom

empagliflozin and dapagliflozin were exchanged for one another at the same daily dose of 10 mg. The NT-proBNP concentration measured in the same patient during treatment with each of the molecules did not differ (1730 ± 1638 versus 1613 ± 1514 pg/ml; $p = 0.146$), nor did body weight, blood pressure, heart rate, eGFR or glycated hemoglobin percentage. Over one year of follow-up, in which patients remained on the molecule they had chosen themselves after the exchange had been completed, survival free of heart failure exacerbation and death from any cause did not differ ($p = 0.898$) [25]. On hard endpoints, by contrast, the picture is divergent. In a nationwide cohort of 72,752 propensity-matched pairs of patients with type 2 diabetes, the primary composite endpoint did not differentiate the molecules (HR 0.93; $p = 0.07$). A lower risk of heart failure-related events (HR 0.84; 95% CI 0.714–0.989) and of cardiovascular death (HR 0.76; 95% CI 0.618–0.921) was, however, observed in those receiving dapagliflozin [26]. In a cohort of 11,007 pairs of patients with diagnosed heart failure the direction was the opposite: a lower risk of the composite endpoint of death from any cause or hospitalization was seen in those receiving empagliflozin (HR 0.90; 95% CI 0.86–0.94), the difference arising from hospitalizations, with no difference in all-cause mortality (HR 0.91; 95% CI 0.82–1.00) [27]. Both cohorts are observational, cover different populations and differently defined endpoints, so that the opposite direction of their results does not establish a difference between the molecules but shows that the observational evidence available today does not allow such a determination to be made. Our data add to this picture a biomarker layer in patients with atrial fibrillation, with the same daily dose in all treated patients and with sampling performed in the same phase of the dosing interval.

The absence of a detectable difference in our cohort is compatible with, but does not establish, a shared class-level biomarker pattern. With group sizes of 98 and 70 patients and 80% power, a difference of approximately 0.44 standard deviations was detectable; moderate and large differences are therefore unlikely, but small differences cannot be excluded. Within this resolution, the data did not identify a biomarker-based reason to prefer one molecule over the other. Clinical selection should continue to rely on established indications, kidney function, comorbidities, treatment tolerance and patient factors rather than on these biomarker findings. Current guidelines formulate recommendations at the class level across the range of ejection fraction and list dapagliflozin and empagliflozin without differentiating their class of recommendation or level of evidence [8]. Preclinical findings provide some support for shared mechanisms: inhibition of the sodium-hydrogen exchanger and lowering of cytosolic sodium have been demonstrated in mouse cardiomyocytes for empagliflozin, dapagliflozin and canagliflozin [30]. This does not imply identical functional effects in every model. In a rat model of heart failure with preserved ejection fraction, dapagliflozin acted directly on exchanger activity in endothelial cells, whereas no direct action was found in isolated cardiomyocytes [31]. Some experimental comparisons have distinguished canagliflozin, which is less selective for SGLT2; in those models, empagliflozin and dapagliflozin produced similar readouts [12].

4.5. Direction of the association and confounding by indication

The direction of the endothelial observations deserves emphasis, because it is opposite to the pleiotropic hypothesis in its usual formulation. Treated patients had higher, not lower, sVCAM-1 concentrations. This is the picture that would be expected if this analyte reflects the cardiovascular disease burden that prompted the drug to be prescribed. In symptomatic heart failure, irrespective of ejection fraction, SGLT2 inhibitors carry a class I recommendation and level of evidence A [8]. In an observational cohort, the fact of being treated is therefore in part a marker of having this disease. The clinical differences documented in Table 1 are of a magnitude that makes this interpretation difficult to avoid, and its sharpest single expression is the distribution of functional class: all patients in NYHA class III were in the treated group, and among the 41 patients with heart failure not receiving an SGLT2 inhibitor there was not a single one. The halving of the association for sVCAM-1 after adjustment for eight clinical variables, from 25.9% to 12.8%, shows quantitatively how large a part of the observed difference is explained by the severity of disease. The analysis restricted to NYHA class II, in which both groups were represented, addresses this observation directly: after removal of the patients in class III the association persisted (+ 15.7%; 95% CI + 2.5 to + 30.7), with a point estimate slightly higher than in the whole cohort and a correspondingly wider confidence interval.

Concomitant pharmacotherapy requires separate consideration, because its possible influence acts in the direction opposite to the difference observed. In the biomarker substudy of the DAPA-HF trial, patients receiving renin-angiotensin system blocking drugs had a lower VCAM-1 concentration than those not receiving them (1036.1 ± 351.1 versus 1125 ± 455.6 ng/ml; $p < 0.001$), and an analogous, though smaller, difference was recorded for statins (1035.7 ± 359.2 versus 1064.9 ± 372.7 ng/ml; $p = 0.04$) [32]. In our cohort both of these therapies were markedly more frequent in patients receiving an SGLT2 inhibitor: renin-angiotensin system blockade was received by 91.8% of patients treated with empagliflozin and 95.7% of those treated with dapagliflozin versus 73.1% of those not receiving an SGLT2 inhibitor (both $p < 0.001$), and a statin by 84.7% and 88.6% versus 68.1%, respectively ($p = 0.005$ and $p = 0.002$). If, therefore, the difference in sVCAM-1 concentration were to arise from differing concomitant pharmacotherapy, its expected direction would be the reverse of that observed: more intensive treatment in the group receiving an SGLT2 inhibitor should reduce this difference rather than generate it. This observation does not determine the nature of the association and does not exclude confounding by factors not taken into account, but it does remove one of the competing explanations, as does the analysis restricted to NYHA class II. The comparison is, moreover, subject to the reservation that definitions of renin-angiotensin system blockade may differ between studies, and the work cited did not state whether it included an angiotensin receptor-neprilysin inhibitor.

This direction is not, however, isolated in the literature. In the biomarker substudy of the DAPA-HF trial, covering 3051 patients with heart failure and reduced ejection fraction, fifty-two weeks of dapagliflozin did not change the concentration of circulating VCAM-1: the placebo-corrected change was -7.18 ng/ml (95% CI -32.7 to $+18.4$; $p = 0.58$) [32]. The same absence of change has been described outside heart failure and under conditions in which endothelial function improved. In a randomized trial in patients with type 2 diabetes with asymptomatic carotid atherosclerosis, twelve weeks of dapagliflozin increased flow-mediated dilation of the brachial artery and the nitrite concentration without changing the

concentrations of the soluble adhesion molecules VCAM-1 and ICAM-1 [14]. In a prospective study in patients with type 2 diabetes, in which empagliflozin was given at a dose of 10 mg daily for twelve weeks, the plasma VCAM-1 concentration increased significantly, with a concurrent fall in ICAM-1 and no change in E-selectin [33]. That study, however, covered eleven patients, and biomarker measurements were performed in nine, without randomization and without a control group, so that its weight is incomparably smaller than that of randomized material. None of these works demonstrated a reduction in circulating VCAM-1 under an SGLT2 inhibitor, and the cross-sectional difference we observed therefore does not stand in contradiction to the prospective evidence available.

A reading that links the profile observed with the indication rather than with the molecule itself is indirectly supported by the result of a randomized trial. In patients with persistent atrial fibrillation free of diabetes, heart failure and chronic kidney disease, three months of dapagliflozin after ablation changed neither arrhythmia burden (7.5% versus 8.1%; $p = 0.48$) nor left atrial dimension ($p = 0.87$). The authors concluded that a short-term direct antiarrhythmic action of dapagliflozin in this population was not confirmed and that the favorable signals observed previously in patients with a cardiometabolic burden may in part reflect an influence on comorbidities [24]. The cohort we studied is a cohort defined by the presence of such an indication.

Nothing in these data indicates that SGLT2 inhibition intensifies endothelial activation. On the contrary – in a non-diabetic rat model of heart failure with preserved ejection fraction, six weeks of dapagliflozin reversed endothelial activation and the deficit of endothelial nitric oxide synthase, with blood pressure remaining markedly elevated [31]. The authors of the DAPA-HF substudy juxtaposed their negative result in the same way: the absence of a change in the circulating concentration against an earlier report of a reduction by empagliflozin of VCAM-1 expression in myocardial preparations obtained from patients with heart failure with preserved ejection fraction, obtained under in vitro conditions [32]. Three levels of evidence therefore form a coherent order: reversal of endothelial activation under experimentally controlled exposure, absence of a change in the circulating VCAM-1 concentration under randomization in humans [32], and a higher circulating concentration in our cross-sectional cohort, in which treatment assignment was determined by the indication. The divergence therefore concerns the compartment of measurement and the study design, not the direction of the drug's action. The divergence between the two compartments is, moreover, documented experimentally: in cultured human endothelial cells the concentration of the soluble molecule VCAM-1 in the medium did not, under all conditions, reflect its expression on the cell surface, so that measurement of the soluble form need not be a measure of the state of the endothelial surface [34]. What the data do indicate is something else: endothelial activation remains detectable and remains graded with the severity of disease also in patients receiving contemporary guideline-directed therapy, including an SGLT2 inhibitor. This reading is supported by the observation that, in the prospective study cited, a higher VCAM-1 concentration was accompanied by an improvement, rather than a worsening, of the vascular response to insulin and of skeletal muscle and myocardial microvascular perfusion [33]. The practical reading is as follows: if residual endothelial activation persists despite full guideline-directed pharmacotherapy, adhesion molecule concentrations may indicate a component of vascular risk that is captured neither by natriuretic peptides nor by ejection

fraction. Whether this component is modifiable and whether it carries prognostic information beyond established markers cannot be determined by a cross-sectional design.

The severity of disease does not, however, exhaust the observations. Both clinical reference markers behaved exactly as a marker of disease burden behaves: their large crude differences were abolished entirely both by adjustment for clinical variables and by restriction of the analysis to a single functional class. The difference for sVCAM-1 did not behave in this way in either of these analyses. Such behavior has an external counterpart: in the DAPA-HF substudy cited, the VCAM-1 concentration remained independently associated with prognosis after NT-proBNP, high-sensitivity troponin T and C-reactive protein had been taken into account, and it correlated only moderately with the first two (Pearson correlation coefficients 0.28 and 0.21, respectively) [32]. In the same substudy, tertiles of VCAM-1 concentration did not differ in ejection fraction, duration of heart failure or previous hospitalization for it, whereas the frequency of atrial fibrillation was almost twice as high in the highest tertile as in the lowest (52.0% versus 27.5%) [32]. The absence of an ejection fraction gradient is not, moreover, at odds with the correlation we observed, because that study enrolled only patients with an ejection fraction not exceeding 40%, so that the range of its variability there was considerably narrower than in our cohort. An analogous pattern has been described outside heart failure. In a population-based cohort followed for twenty years, the soluble molecule VCAM-1 was, after adjustment for age and sex, the only one of thirteen circulating inflammatory markers measured to be associated with incident atrial fibrillation. The association persisted after adjustment for NT-proBNP and high-sensitivity troponin T (hazard ratio per standard deviation 1.34; 95% CI 1.11–1.62), with a strength at least equal to that of the association for the natriuretic peptide itself (1.15; 95% CI 1.04–1.26). Markers of systemic inflammation, including high-sensitivity C-reactive protein, showed no association [21]. NT-proBNP and troponin T should not, however, be called formal negative controls, because their concentrations cannot be assumed a priori to be insensitive to treatment. Their behavior does, nevertheless, set the background against which the persistence of the difference for sVCAM-1 acquires meaning, and it indicates that the set of covariates adopted captures a substantial part of the differences in disease severity separating the groups compared. The direction of possible confounding by concomitant therapy argues, moreover, against rather than for the difference observed: in the DAPA-HF substudy cited, lower VCAM-1 concentrations were found in patients receiving a statin and renin-angiotensin system blocking drugs [32], and in our cohort a statin was received by 84.7% and 88.6% of patients receiving an SGLT2 inhibitor versus 68.1% of those not receiving one. If this component of care influenced the sVCAM-1 concentration, it would act in the treated group in the direction of lowering it.

4.6. Results for the oxidative domain and the integrative marker

The concentrations of both markers of the oxidative domain did not differentiate the groups either in the crude comparison or in the adjusted model. Angiopoietin-2 and GDF-15 differentiated the groups in the crude comparison, but these differences disappeared entirely after adjustment for clinical variables ($p = 0.930$ and 0.951). The behavior of the two analytes was, moreover, not the same for both molecules: in

the crude comparison the difference from the untreated group reached the significance level adopted for angiotensin-2 only with empagliflozin and for GDF-15 only with dapagliflozin (Table 2). Because the direct comparison of the two molecules showed no difference for either of these analytes, and because after adjustment for clinical variables both lost their association with exposure, this divergence should not be read as a difference between the molecules. This is behavior identical to that of NT-proBNP and troponin T, whose differences the same set of variables likewise abolished entirely. It points to a differing disease burden of the groups compared rather than to exposure to an SGLT2 inhibitor. In the case of GDF-15 this is consistent with its biologically non-specific character: this marker is strongly induced under conditions of stress and inflammation, and in experimental models its increase has been linked with myocardial injury and pressure overload [20]. It should therefore not be interpreted as a readout of a single pathophysiological axis. A pharmacological reading of the crude difference in GDF-15 concentration is, moreover, contradicted by the direction observed under randomization: in the EMPEROR program, empagliflozin was associated at week 52 with a small increase, rather than a fall, in the concentration of this analyte (ratio of relative change 1.08; 95% CI 1.01–1.15; $p = 0.02$) [35]. At the same time, the width of the confidence interval for GDF-15 after adjustment, from -48.9% to $+87.8\%$, reflects the very large variability of the concentrations of this analyte in the cohort studied and calls for caution in treating its result on a par with the others. We present the results for both analytes for completeness and not as observations in their own right; their behavior is also discussed in Section 4.3.

For 8-hydroxy-2'-deoxyguanosine there is randomized evidence that requires juxtaposition with the null result above. A randomized trial with blinded endpoint assessment enrolled 80 patients with early-stage type 2 diabetes, patients with chronic atrial fibrillation being excluded. Sixteen weeks of adding dapagliflozin to metformin was associated in that trial with a lower urinary concentration of this analyte, normalized to creatinine, than increasing the dose of metformin alone (4.0 ± 1.9 versus 5.8 ± 2.3 ng/mg creatinine; $p < 0.001$) [36]. The structure of this difference deserves attention. It arose from a fall of 0.6 ± 1.8 ng/mg creatinine in the dapagliflozin arm ($p = 0.047$) and a rise of 1.1 ± 2.2 in the comparator arm ($p = 0.004$). In the subgroup with a baseline glycated hemoglobin of at least 7.0%, that is the same subgroup in which flow-mediated dilation of the brachial artery differed between the arms in favor of dapagliflozin, the concentration in the dapagliflozin arm did not change significantly ($p = 0.66$), whereas in the comparator arm it increased ($p = 0.031$) [36]. The measurements were, moreover, performed in urine rather than in plasma, and urinary excretion and circulating concentration are not the same quantity. The interventional data in this domain are not, however, unidirectional. An open-label study allocated 85 patients with type 2 diabetes according to the last digit of the hospital number, and a per-protocol analysis was performed in 76 of them (imeglimin 34, empagliflozin 42). After 24 weeks the urinary 8-hydroxy-2'-deoxyguanosine concentration increased in the empagliflozin arm from 12.6 [8.8–15.1] to 20.0 [14.2–26.0] $\mu\text{g/g}$ creatinine ($p < 0.001$). In parallel, metabolites of reactive oxygen species and the total antioxidant potential increased, and soluble tumor necrosis factor receptors 1 and 2 decreased (all $p \leq 0.002$) [37]. Changes were observed in both arms. In unadjusted between-group comparisons no significant differences were shown for the redox and inflammatory markers analyzed (p from 0.138 to 0.578), yet in a separate multivariable model allocation to the empagliflozin group was

independently associated with a greater increase in urinary 8-OHdG excretion ($\beta = 7.78$; 95% CI 1.82–13.73; $p = 0.011$) [37]. The authors interpreted the concurrent increase in markers of oxidative stress and in total antioxidant potential as a possible hormetic response; they did not, however, measure mitochondrial oxygen species or mitokines, and they indicated the open-label nature of the trial and the predictable method of allocation as limitations [37]. What matters for the reading of our result is that, with a favorable direction of change in markers of inflammation and arterial stiffness, the analytes of oxidative damage did not fall but rose. The absence of a difference in this domain is therefore not in itself an argument against the presence of an endothelial effect. In interventional studies in which analytes of the oxidative domain were measured under an SGLT2 inhibitor, patients with atrial fibrillation were either excluded [36] or their participation was not reported [37]; in work [14] atrial fibrillation did not figure among the exclusion criteria.

Juxtaposing the two domains with tissue data indicates where the boundary between them may run. In 80 right atrial appendages obtained during coronary artery bypass grafting or aortic valve replacement, among them from 18 patients with a history of atrial fibrillation, samples with tumor necrosis factor α expression above the median were characterized by lower expression of endothelial nitric oxide synthase and higher expression of SGLT2, NADPH oxidase 2 and VCAM-1. All the samples from patients with a history of atrial fibrillation belonged to that very group [38]. The transporter itself co-localized in highly inflamed tissue with the endothelial marker CD31 and with the proinflammatory macrophage marker CD86, and in cultured human coronary artery endothelial cells tumor necrosis factor α simultaneously increased the expression of SGLT2 and of VCAM-1 [38]. Two readings for our data follow from this. First, the coupling between the oxidative signal and endothelial activation was described there at the tissue level, which is in agreement with the divergence we observed between the oxidative domain measured systemically and the adhesion marker. Second, the expression of the transporter itself is not a fixed quantity and increased with the intensity of the inflammatory response, which bears on the question of the direction of the association raised above: patients receiving an SGLT2 inhibitor had in our cohort a higher interleukin 6 concentration (Table 1), although we did not measure tissue expression. Two things cannot, by contrast, be read from that work. VCAM-1 was not among the markers additionally elevated in patients with atrial fibrillation within the highly inflamed group. And attenuation of induced VCAM-1 expression in endothelial cells was demonstrated for empagliflozin used together with semaglutide, not for SGLT2 inhibition in monotherapy, at concentrations lower than typical plasma concentrations [38].

The pattern in which the adhesion marker retains an association with exposure while the reference markers lose it can be placed in a broader conceptual frame. A consensus statement of the European, American, Asia-Pacific and Latin American societies defines atrial cardiomyopathy as a complex of structural, architectural, contractile or electrophysiological changes of the atria capable of leading to clinically relevant manifestations, including atrial fibrillation. At the same time it emphasizes that atrial cardiomyopathy is to be distinguished from the arrhythmia itself: it may constitute a substrate for atrial fibrillation, and the arrhythmia may subsequently accelerate its progression [39]. In such a framing, the analytes that in our cohort lost their association with exposure after adjustment are a readout of the

severity of this substrate and not a readout of the drug's action. Observations from human material are consistent with this: in a group of 117 patients undergoing cardiac surgery, VCAM-1 and GDF-15 concentrations were significantly higher in patients with permanent atrial fibrillation than in patients without fibrillation, and the arrhythmic phenotypes were accompanied by greater cardiomyocyte apoptosis and lower, semi-quantitatively assessed, connexin 43 expression [40]. This comparison has additional value for us, because the work cited covered five analytes also measured in our cohort, among them VCAM-1 and GDF-15, which belong to our panel. Absolute VCAM-1 concentrations should not, by contrast, be compared directly between studies. The tissue material there came, moreover, from the right appendage, and patients with permanent fibrillation constituted the reference group for postoperative fibrillation rather than the object of the study, so that this comparison indicates the direction and not the magnitude of the effect.

4.7. Strengths of the study

Two features of the cohort strengthen what can be inferred from it. First, the exclusion criteria removed a substantial part of the common extracardiac causes of endothelial activation and of increased oxidative stress: active infection, fever, recent coronary syndrome and stroke, recent decompensation, surgery, bleeding, malignancy, autoimmune disease requiring steroid or immunosuppressive therapy, uncontrolled thyroid disease, impaired liver function, glucocorticoids, non-steroidal anti-inflammatory drugs and antiplatelet drugs. Few biomarker cohorts from clinical practice can say this of themselves, and it substantially limits the number of alternative explanations for the differences observed.

Second, blood sampling was planned with respect to pharmacotherapy and not to the time of day alone, so that every patient was studied before the morning dose, in the same phase of the dosing interval of the SGLT2 inhibitor. The requirement of at least three months of uninterrupted treatment limited the possibility of classifying as exposed those patients who had recently started therapy. The same applied to the oral anticoagulant, taken in the morning by every patient, so that sampling was performed pre-dose with respect to both drugs. This matters because trough and peak concentrations of direct oral anticoagulants differ approximately twofold in real-life atrial fibrillation populations [41], and because exposure is further modified by intake relative to food and by co-ingested dietary constituents [42]; a uniform pre-dose window keeps this component of the pharmacological context constant across the cohort. Pre-analytical handling, the distribution of samples across plates, calibration and operator assignment were uniform throughout the series, with precision below 5% within an assay and below 10% between assays. Complete measurements of the six panel markers were obtained in all 287 participants, so that the primary analysis did not require imputation.

5. Limitations

The design is cross-sectional, and this is a fundamental limitation. Biomarkers were measured once, without values from before the start of treatment, so that no statement about change over time or about a causal relationship is possible; the associational formulation of every result in this work is a consequence of this.

Treatment was not allocated at random, and its assignment was strongly linked with the indication. Patients receiving an SGLT2 inhibitor had a significantly lower ejection fraction, more frequent heart failure and more intensive concomitant therapy than those not receiving one, and all patients in NYHA class III were in the treated group; confounding of this magnitude is not removed in its entirety by adjustment for measured variables. The analyses performed limit this problem but do not abolish it: the association persisted in every model specification and after restriction of the analysis to a single functional class, and both clinical reference markers lost their association with exposure in these same analyses. Residual confounding nevertheless remains possible, and it cannot be determined whether the pattern observed is determined by exposure itself or by features of care coexisting with it. Data on smoking were not collected, and it therefore remains an unmeasured confounder for all the analytes measured.

All patients receiving an SGLT2 inhibitor were given the same daily dose, so that an assessment of the dose-response relationship could not be carried out. The within-class comparison had the power to detect a difference of the order of 0.44 standard deviations, so that small differences between empagliflozin and dapagliflozin cannot be excluded. The Holm correction across the six panel markers was applied to the p values from the adjusted models; only the result for sVCAM-1 met this criterion. The remaining comparisons, including the exploratory analyses presented in Table S2, should be treated as hypothesis-generating.

Lipid peroxidation was not measured; 8-isoprostaglandin F2 α remains to be measured, so that the oxidative domain is represented by two analytes and cannot be regarded as exhaustively characterized. This gap is not filled by the analytes measured. In the work in which advanced oxidation protein products were first described, their plasma concentrations in patients with chronic renal failure correlated with dityrosine and AGE-pentosidine but did not correlate with thiobarbituric acid-reactive substances, which indicates that this analyte does not inform about lipid peroxidation [19]. In a randomized trial in which free 8-isoprostan was measured in patients receiving dapagliflozin, its concentration did not differ from the control group [14]; this observation cannot, however, be transferred either to the remaining analytes of the oxidative domain or to our cohort. Plasma was collected on sodium heparin, so that absolute concentrations may not be directly comparable with studies using serum or EDTA plasma. This limitation does not concern our material alone: for sVCAM-1 there is a lack of harmonized cut-off values and of full standardization of measurement, which limits the direct comparison of absolute concentrations between studies [15]. The study was conducted at a single center, in a single population, without clinical endpoints and without external validation. Whether sVCAM-1 adds information to the risk assessment already available in atrial fibrillation treated with oral anticoagulation — in which established clinical scores discriminate mortality only moderately and models built on routinely collected variables perform substantially better [43] — cannot be assessed here, because no outcomes were recorded. The result requires independent confirmation in a separate cohort.

6. Conclusions

In patients with non-valvular atrial fibrillation, endothelial but not oxidative biomarker profiles differed according to SGLT2 inhibitor exposure, although sVCAM-1 was the only analyte independently associated with exposure after multivariable adjustment and correction for multiple testing. Empagliflozin and dapagliflozin were not statistically distinguishable across the eight measured analytes, but small between-drug differences cannot be excluded. Treated and untreated patients differed substantially in cardiovascular disease severity. The sVCAM-1 association persisted across sensitivity analyses, including restriction to NYHA class II, whereas crude differences in NT-proBNP and hs-TnT disappeared after adjustment. Residual confounding remains possible, and the cross-sectional design does not permit causal inference. Longitudinal studies with biomarker measurements before and after treatment initiation are needed to determine whether sVCAM-1 reflects SGLT2 inhibitor exposure, underlying disease or both.

Abbreviations

AOPP, advanced oxidation protein products; eGFR, estimated glomerular filtration rate; GDF-15, growth differentiation factor 15; hsCRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; SGLT2, sodium-glucose co-transporter 2; sE-selectin, soluble E-selectin; sVCAM-1, soluble vascular cell adhesion molecule 1; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

Declarations

Ethics approval and consent to participate

The study was approved by the Bioethics Committee at Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (approval no. KB 295/2024). All participants provided written informed consent before enrollment.

Consent for publication

Not applicable.

Availability of data and materials

The de-identified dataset supporting the conclusions of this article is not publicly available because of institutional and legal restrictions on sharing individual clinical data. The data are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: Ł. Wołowiec and G. Grześk. Methodology: Ł. Wołowiec, A. Wołowiec and M. Kozakiewicz. Formal analysis: Ł. Wołowiec and J. Budzyński. Investigation: Ł. Wołowiec, J. Banach, A. Szymczak, M. Janiak, A. Wołowiec, M. Kozakiewicz and J. Warecka. Resources: Ł. Wołowiec and J. Banach. Data curation: Ł. Wołowiec, A. Szymczak, M. Janiak and J. Warecka. Validation: A. Wołowiec. Visualization: M. Janiak. Writing - original draft: Ł. Wołowiec, J. Banach and G. Grześk. Writing - review and editing: all authors. Supervision: Ł. Wołowiec and G. Grześk. Project administration: Ł. Wołowiec. All authors read and approved the final manuscript.

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None.

Related work from the same cohort

This analysis is based on the same prospective single-center registry as the companion manuscript on oral anticoagulant class and oxidative DNA damage (Wołowiec et al., submitted). The studies address different exposures, primary endpoints and hypotheses. The companion manuscript compares direct thrombin inhibition with factor Xa inhibition in 266 patients receiving a direct oral anticoagulant, whereas this study evaluates SGLT2 inhibitor exposure in the full cohort of 287 patients. Cohort characteristics and pre-analytical and laboratory methods are shared and are reported consistently.

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Figures

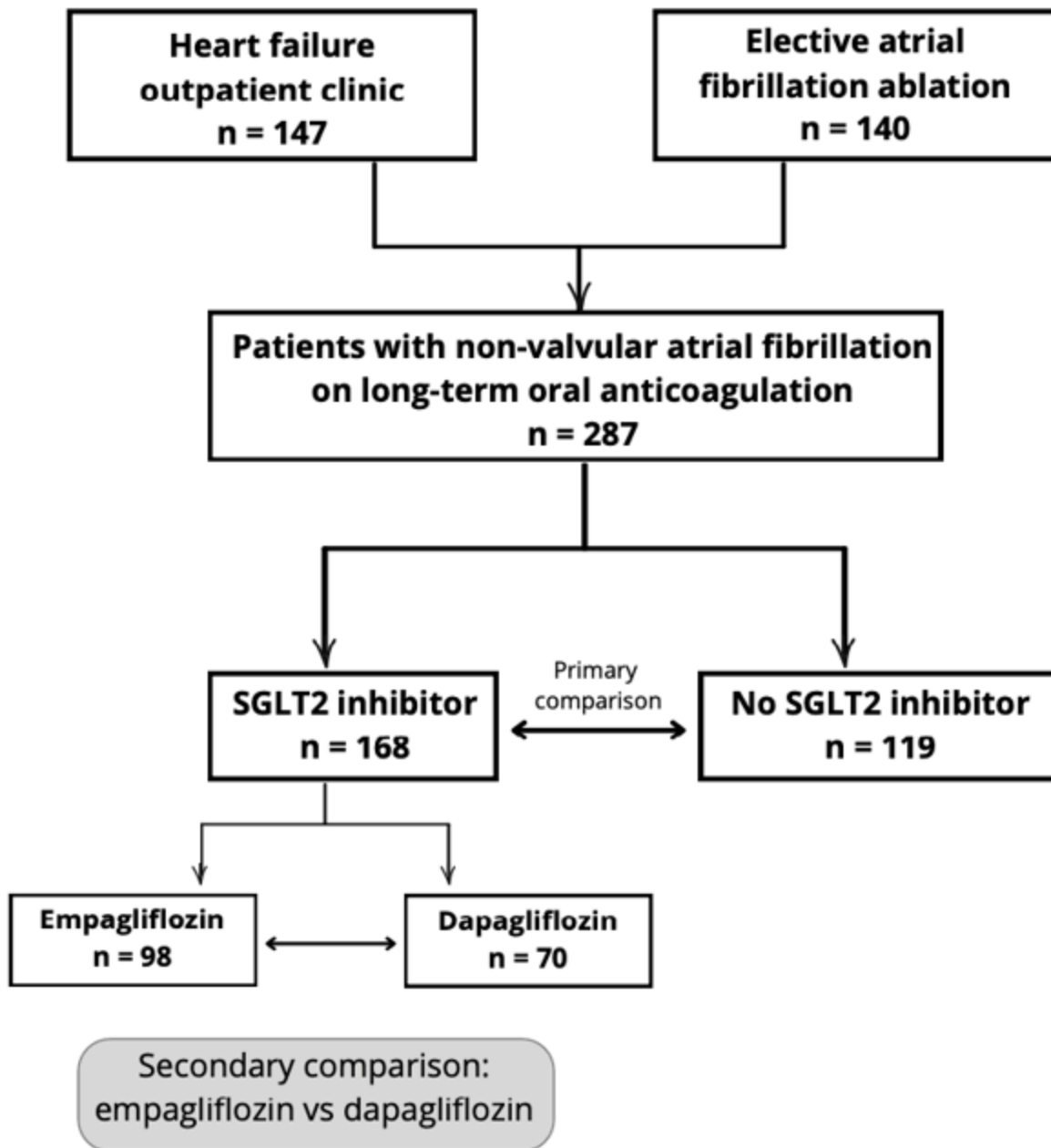


Figure 1

Cohort structure and SGLT2 inhibitor exposure groups

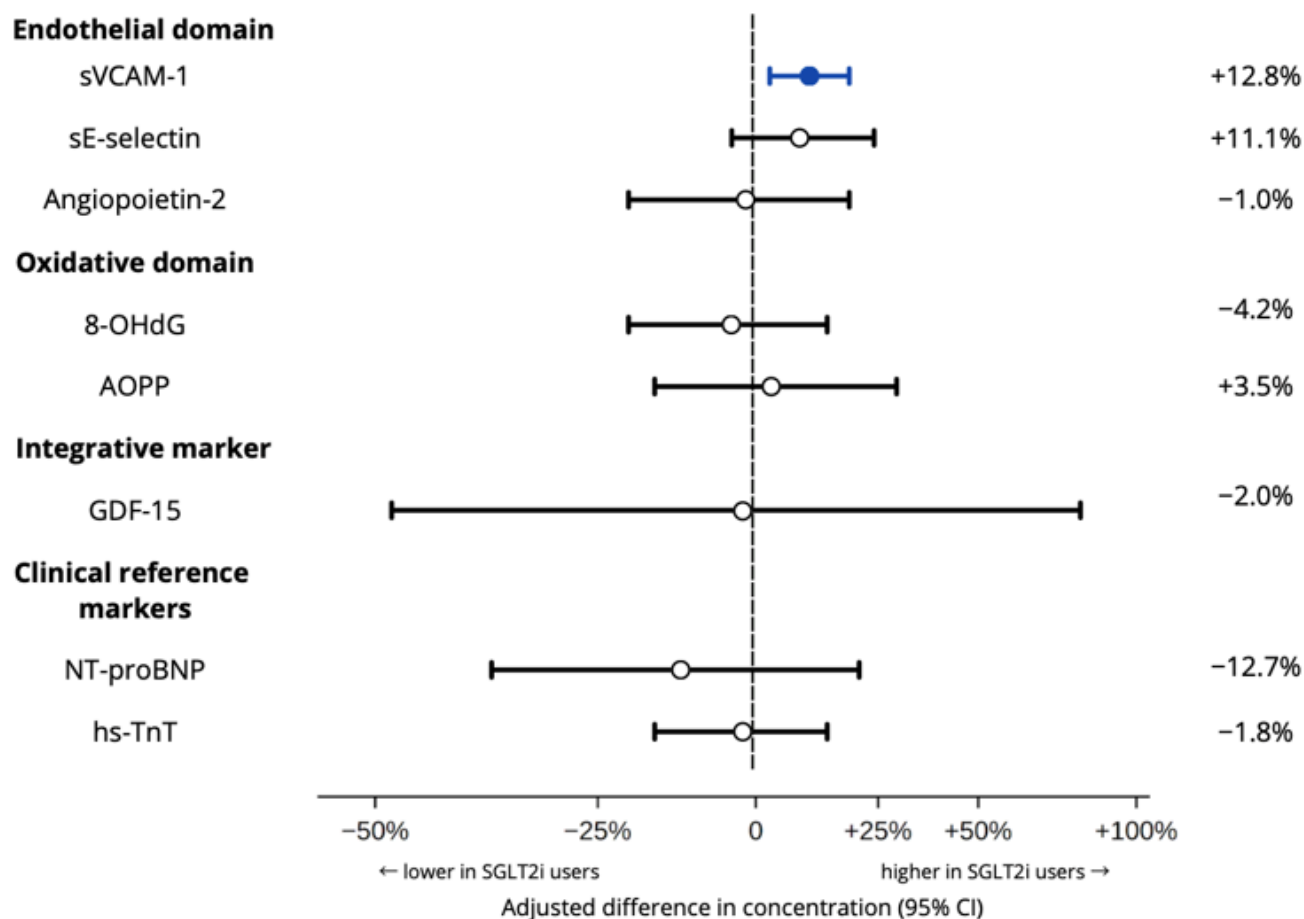


Figure 2

Adjusted analyte differences according to SGLT2 inhibitor exposure

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