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Overall and inter-individual effect of four different drug classes on soluble urokinase plasminogen activator receptor in type 1 and type 2 diabetes

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Abstract

Aim: To evaluate the effect of four different drug classes on soluble urokinase plasminogen activator receptor (suPAR), a biomarker active in multiple inflammatory processes and a risk factor for complications, in people with type 1 and type 2 diabetes.

Methods: We conducted post hoc analyses of a randomized, open-label, crossover trial including 26 adults with type 1 and 40 with type 2 diabetes with urinary albumin-creatinine ratio ≥30 and ≤500 mg/g assigned to 4-week treatments with telmisartan 80 mg, empagliflozin 10 mg, linagliptin 5 mg and baricitinib 2 mg, separated by 4-week washouts. Plasma suPAR was measured before and after each treatment. SuPAR change after each treatment was calculated and, for each individual, the best suPAR-reducing drug was identified. Subsequently, the effect of the best individual drug was compared against the mean of the other three drugs. Repeated-measures linear mixed-effects models were employed.

Results: The baseline median (interquartile range) plasma suPAR was 3.5 (2.9, 4.3) ng/mL. No overall effect on suPAR levels was observed for any one drug. The individual best-performing drug varied, with baricitinib being selected for 20 participants (30%), followed by empagliflozin for 19 (29%), linagliptin for 16 (24%) and telmisartan for 11 (17%). The individual best-performing drug reduced suPAR by 13.3% (95% confidence interval [CI] 3.7, 22.8; $P = 0.007$). The difference in suPAR response between the individual best-performing drug and the other three was −19.7% (95% CI −23.1, −16.3; $P < 0.001$).

Conclusions: We demonstrated no overall effect of 4-week treatment with telmisartan, empagliflozin, linagliptin or baricitinib on suPAR. However, individualization of treatment might significantly reduce suPAR levels.

KEYWORDS

albuminuria, angiotensin receptor blocker, baricitinib, chronic kidney disease, dipeptidyl peptidase-4 inhibitor, inflammation, JAK–STAT inhibitor, personalized medicine, SGLT2 inhibitor

1 | INTRODUCTION

Chronic kidney disease (CKD) in diabetes is a long-term complication which is not only the leading cause of kidney failure in developed countries but is also greatly associated with substantially increased risk of cardiovascular disease and death.[1–3] Recent advances in treatment of CKD have been driven by novel drug classes exhibiting kidney protective effects[4–6] and, in parallel, the search for early biomarkers of CKD or renal damage has been intensified.

Higher circulating levels of soluble urokinase plasminogen activator receptor (suPAR) have been increasingly shown to be associated with various pathological conditions in individuals both with and without diabetes, such as CKD, cardiovascular disease, malignancies and mortality. Furthermore, a recent genome-wide association meta-analysis including over 25 000 individuals indicated a possible causal effect of increased suPAR levels in atherosclerotic processes,[7] further implying its importance.

One previous study has investigated the effect of the sodium-glucose cotransporter 2 (SGLT2) inhibitor, dapagliflozin, on suPAR levels in an acute setting and after 12 weeks of treatment.[8] In that study, no overall effect of the treatment was found, despite the reports of possible anti-inflammatory effects of SGLT2 inhibitors seen in other studies.[9,10]

The effects of kidney protective medications on urinary albumin excretion, the "gold standard" marker of early kidney damage in diabetes, have been shown to be highly variable.[11,12] We have demonstrated that by rotation through different drug classes, single-drug treatment of elevated urinary albumin-creatinine ratio (UACR) can be significantly optimized for an individual with diabetes.[13] The aim of this study was to investigate whether there was a similar individualized effect of suPAR-lowering response after treatment with telmisartan (angiotensin receptor blocker), empagliflozin (SGLT2 inhibitor), linagliptin (dipeptidyl peptidase-4 inhibitor) and baricitinib (Janus kinase inhibitor), in a rotational randomized crossover trial in type 1 and type 2 diabetes, and also whether the response was correlated to UACR-lowering response.

2 | METHODS

2.1 | Study design

This study was based on post hoc analyses of the ROTATE trials, two randomized, prospective, open-label, multicentre, crossover trials including participants with type 1 and type 2 diabetes (ROTATE-1 and ROTATE-2, respectively), conducted between February 2017 and October 2019 at Steno Diabetes Center Copenhagen (Herlev, Denmark), Ziekenhuis Groep Twente (Almelo, the Netherlands) and Bethesda Diabetes Research Center (Hoogeveen, the Netherlands). The aim of those trials was to assess the individual UACR-lowering responses of telmisartan, empagliflozin, linagliptin and baricitinib in participants with diabetes and elevated UACR. The results showed highly individual effects on UACR across participants, both in terms of magnitude of effect as well as which drug entailed the greatest effect.[13] All participants gave written informed consent, and the trials were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The trials were registered at clinicaltrialregister.nl (NTR5602, NTR5603) and the European Union Trials Register (2015-005691-26, 2017-004641-25).

2.2 | Participants

Inclusion and exclusion criteria have been described in full before.[13] In short, adults with type 1 or type 2 diabetes, a UACR between 30 and 500 mg/g, and an estimated glomerular filtration rate (eGFR) ≥ 45 mL/min/1.73 m^2 were eligible for inclusion. Individuals with a cardiovascular event within 6 months of screening or prescribed treatment with a glucagon-like peptide-1 receptor agonist were excluded. Treatment with renin-angiotensin-aldosterone system (RAAS) blockers, SGLT2 inhibitors or dipeptidyl peptidase-4 inhibitors was discontinued at screening and not allowed throughout the study period.

2.3 | Intervention and blinding

In random order, participants received 4-week treatments with an angiotensin receptor blocker (telmisartan 80 mg), an SGLT2 inhibitor (empagliflozin 10 mg), a dipeptidyl peptidase-4 inhibitor (linagliptin 5 mg) and a Janus kinase inhibitor (baricitinib 2 mg), with 4-week washout periods in-between each treatment. Treatment sequence randomization was carried out by computer-generated randomized code supplied by the coordinating centre. Participants and investigators were not blinded because the primary aim of the study was assessment of intra-individual response and thus systemic bias was avoided.

2.4 | Procedures

After screening, participants entered a run-in period in which use of RAAS, SGLT2 and dipeptidyl peptidase-4 inhibitors was discontinued. During this period, blood pressure and glycated haemoglobin (HbA1c) levels were monitored and stabilized if they increased by more than 10 mmHg or 5 mmol/mol, respectively, with blood pressure-lowering agents (non-RAAS inhibiting) or glucose-lowering medication (metformin, sulphonylurea derivatives or insulin) at the discretion of the treating physician. If blood pressure and HbA1c could not be stabilized during the maximum 16 weeks duration of the run-in period, the participant was excluded. After the run-in period, participants proceeded to the intervention phase. After the end of the rotation schedule, participants were re-exposed to their individual best UACR-lowering drug for an additional, confirmatory 4-week treatment period.

At each visit, before and after every treatment period, fasting glucose, HbA1c, plasma lipids, liver function variables and creatinine

were measured using standardized methods, and eGFR was calculated using the 2009 CKD Epidemiology Collaboration creatinine-based equation.[14] UACR was measured at each visit in three consecutive first-morning void urine samples by quantifying urinary albumin and creatinine, and the geometric mean of the three measurements at each visit was calculated. SuPAR was measured at each visit using a suPARnostic ELISA (ViroGates, Birkerød, Denmark). For suPAR measurements, EDTA plasma was collected and stored at −80°C until analysis. Plasma suPAR was measured at each visit at the Department of Clinical Research, Copenhagen University Hospital Amager and Hvidovre (Denmark) using a suPARnostic ELISA (ViroGates) in singlets and according to the manufacturer's instructions. To prevent inter-assay variation in suPAR measurements, all samples obtained from each individual participant were analysed on the same plate.

Blood pressure was measured at each visit using a calibrated sphygmanometer, and systolic and diastolic blood pressure were calculated as the mean of three consecutive readings.

2.5 | Outcomes

The co-primary outcomes of the present study were (i) change in suPAR levels between before and after treatment with each of the four drugs and (ii) change in suPAR level after treatment with each individual's best suPAR-lowering drug versus the individual's other three drug treatments. The secondary outcome was the association between baseline suPAR level and best UACR-lowering drug. Tertiary outcomes included correlation between baseline suPAR and UACR levels, as well as correlation between change in suPAR and UACR after each single treatment, and after best suPAR-lowering and UACR-lowering treatments.

2.6 | Statistical analysis

Baseline measurements were obtained at the randomization visit. Normally distributed variables are presented as mean and standard deviation, non-normally distributed variables as median and interquartile range (IQR), and categorical variables as number and percentage. Comparisons between groups with baseline suPAR below or above the median (3.5 ng/mL) were performed using unpaired *t*-tests, Mann-Whitney *U*-tests, and the χ^2 tests, as appropriate. The SuPAR and UACR response for each drug was defined as the difference between start and end of each treatment period calculated on the natural log scale. The primary outcomes were both calculated using repeated-measures linear mixed-effects models with unstructured covariance matrices. The first primary outcome included a single fixed effect for treatment stage for each drug (before/after) and an unstructured covariance matrix that allows for random slopes and intercept. The second primary outcome included interaction between treatment stage (start/end of treatment) and best suPAR-lowering drug (yes/no), and period as fixed effects and an unstructured covariance matrix that allows for random slopes and intercepts for each participant and period. Due to renal excretion affecting suPAR levels, the models were adjusted for eGFR level at each visit, however, to avoid overfitting due to few participants in each group, eGFR was omitted in sub-stratification models for diabetes type. *P* values derived from the primary outcomes were adjusted for multiple testing using the Bonferroni method. The secondary outcome was investigated using logistic regression, and the tertiary outcomes by calculating Pearson correlation coefficients. In all analyses, SuPAR and UACR were transformed on the natural logarithmic scale in order to achieve normal distribution. All statistical analyses were performed using R (R Core Team, version 4.2.1, Vienna, Austria) and RStudio (Rstudio Team, version 2022.07.2 build 576, Boston, Massachusetts). A two-sided *P* value below 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Study participants

From February 2017 until October 2021, 118 individuals were assessed for inclusion in the ROTATE-1 (n = 48) and ROTATE-2 (n = 70) trials. In total, 83 individuals entered run-in, 76 were randomized, and 70 completed the trial (Figure S1). Sixty-six participants had complete suPAR measurements at all visits and were included in the present study. Baseline demographics and laboratory measurements are presented in Table 1, overall and stratified by median baseline suPAR levels. Twenty-six participants had type 1 diabetes and 40 had type 2 diabetes. In total, there were 11 women (17%), the baseline mean ± standard deviation age was 65 ± 10 years, body mass index 30 ± 4 kg/m^2, systolic blood pressure 141 ± 13 mmHg and HbA1c 61 ± 10 mmol/mol, and the median (IQR) C-reactive protein level was 2 (1, 3) mg/L. The median (IQR) baseline suPAR was 3.5 (2.9, 4.3) ng/mL for the overall population, 3.4 (2.9, 3.8) for the type 1 diabetes participants and 3.6 (3.0, 4.3) for the type 2 diabetes participants. When stratifying all participants below/above median baseline suPAR, overall characteristics were comparable, apart from age (61 ± 10 vs. 68 ± 9 years; $P = 0.005$) and eGFR (92 ± 21 vs. 69 ± 19 mL/min/1.73 m^2). Further stratification by the individual best suPAR-lowering drug (Table S1) showed that all characteristics were balanced across best suPAR-lowering drugs, apart from the proportion of participants with type 1 or type 2 diabetes (percentages of participants with type 2 diabetes whose best drug was found to be telmisartan, empagliflozin, linagliptin and baricitinib were 82, 47, 81 and 45, respectively; $P = 0.039$).

3.2 | Drug effect on suPAR

The mean effects of each drug on suPAR levels were all nonsignificant and ranged from −1.4% (95% confidence interval CI −11.8, 9.0%; $P = 0.795$) after treatment with baricitinib to 4.1% (95% CI −4.8, 13.0; $P = 0.370$) after treatment with telmisartan (Table S2, Figure 1). The nonsignificant effects were consistent regardless of diabetes type (Figure S2B).

TABLE 1 Baseline characteristics for the overall population and by stratification for median baseline soluble urokinase plasminogen activator receptor levels

Variable	Overall	Below median suPAR <3.5 ng/mL	Above median suPAR ≥3.5 ng/mL	*P*
n	66	33	33	
Type 2 diabetes, n (%)	40 (60.6)	18 (54.5)	22 (66.7)	0.450
Age, years	64.7 (10.0)	61.3 (9.6)	68.1 (9.2)	0.005
Women, n (%)	11 (17)	4 (12)	7 (21)	0.509
Non-white, n (%)	3 (5)	1 (3)	2 (6)	1.000
Creatinine, µmol/L	88 (27)	77 (24)	98 (25)	0.001
eGFR, mL/min/1.73 m^2	80 (23)	92 (21)	69 (19)	<0.001
Systolic BP, mmHg	141 (13)	142 (13)	140 (13)	0.544
Diastolic BP, mmHg	79 (9)	81 (8)	78 (10)	0.174
Total cholesterol, mmol/L	4.2 (0.9)	4.2 (1.0)	4.1 (0.9)	0.853
LDL cholesterol, mmol/L	2.0 (0.8)	2.0 (0.9)	1.9 (0.6)	0.792
HbA1c, mmol/mol	61 (10)	58 (9)	63 (11)	0.061
C-reactive protein, mg/L	2 [1, 3]	2 [0, 3]	2 [1, 3]	0.154
UACR, mg/g	118 [64, 285]	100 [58, 260]	139 [68, 368]	0.273
Body mass index, kg/m^2	30.0 (4.1)	30.6 (4.4)	29.5 (3.7)	0.269
Smoking, n (%)				0.612
Non-smoker	23 (35)	13 (39)	10 (30)	
Previous	32 (49)	14 (42)	18 (55)	
Current	11 (17)	6 (18)	5 (15)	
History of CV disease, n (%)	21 (32)	9 (27)	12 (36)	0.597

Note: Normally distributed continuous variables are presented as mean (standard deviation) and non-normal as median [interquartile range]. Categorical variables are presented as n (%). *P* values were determined from comparisons of below and above median suPAR, calculated using Student's *t*-test, Mann–Whitney *U*-test and the χ^2 test, as appropriate.

Abbreviations: BP, blood pressure; CV, cardiovascular; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; LDL, low-density lipoprotein; suPAR, soluble urokinase plasminogen activator receptor; UACR, urinary albumin-creatinine ratio.

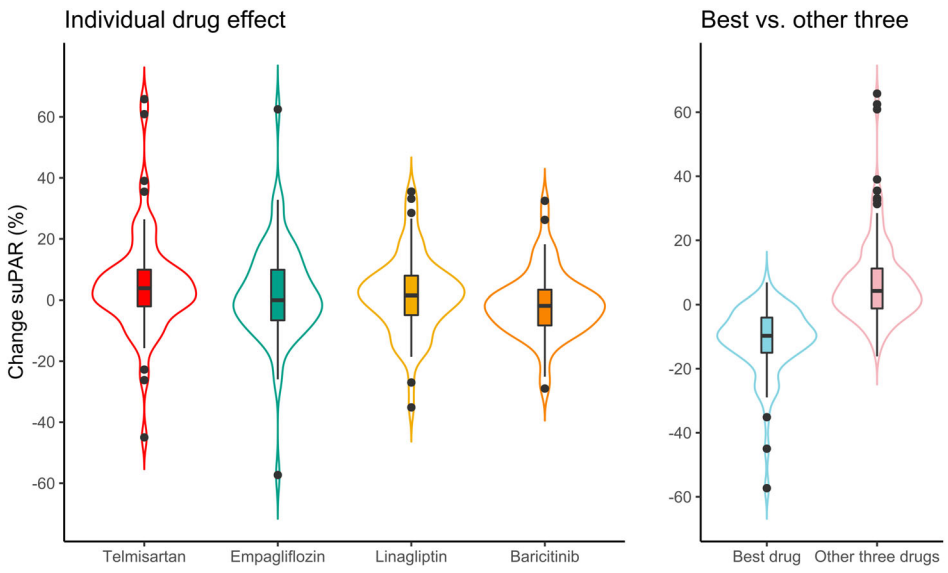

FIGURE 1 Violins with embedded boxplots illustrating soluble urokinase plasminogen activator receptor (suPAR) changes after treatment with telmisartan, empagliflozin, linagliptin and baricitinib and after treatment with the individual best suPAR-lowering drug compared to the other three drugs

Selecting each individual's best suPAR-lowering drug resulted in a mean suPAR change of −13.3% (95% CI −22.9, −3.7%, $P = 0.007$) between start and end of treatment. The change in suPAR during the individual best drug period compared to the mean during the other three drug treatment periods was −19.7% (95% CI −23.1, −16.3%; $P < 0.001$ [Table 2, Figure 1]). Of the

TABLE 2 Estimated differences in soluble urokinase plasminogen activator receptor (suPAR) response after treatment with the individual best suPAR-lowering treatment versus the mean of the other three treatments, as well as with each other drug individually

Comparison	% difference in suPAR response	95% CI	*P*	P_{adj}
Best vs. other three	−19.7	−23.1, −16.3	<0.001	<0.001
Best vs. telmisartan	−21.2	−25.5, −16.8	<0.001	<0.001
Best vs. empagliflozin	−20.7	−25.2, −16.2	<0.001	<0.001
Best vs. linagliptin	−19.6	−24.1, −15.1	<0.001	<0.001
Best vs. baricitinib	−16.2	−21.1, −11.2	<0.001	<0.001
Best UACR-lowering drug vs. other three	−1.3	−6.4, 3.7	0.607	1.000

Note: Furthermore, estimated difference in suPAR response between treatment with the best UACR-lowering drug, compared to the other three treatments.

Abbreviations: CI, confidence interval; P_{adj}, multiple testing Bonferroni-adjusted *P* value; suPAR, soluble urokinase plasminogen activator receptor; UACR, urinary albumin-creatinine ratio.

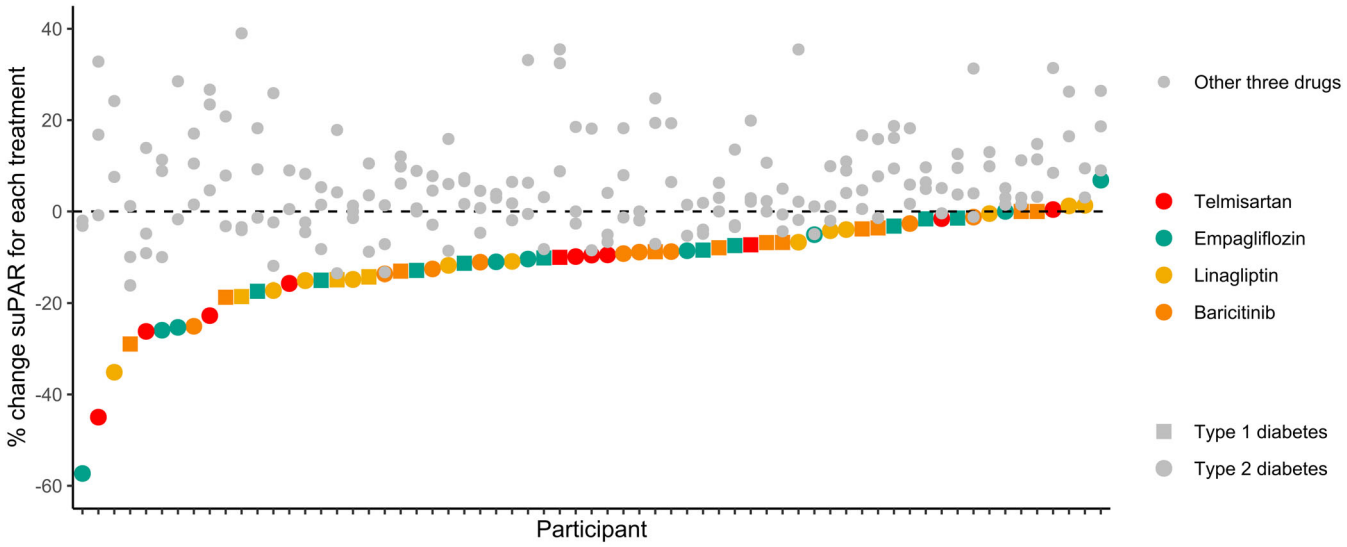

FIGURE 2 Waterfall plot demonstrating the individual participant soluble urokinase plasminogen activator receptor (suPAR) changes for the individual best suPAR-lowering drugs (coloured) and the other three drugs (grey). Squares indicate type 1 diabetes participants, and circles indicate type 2 diabetes participants

66 participants, 11 had telmisartan as the best suPAR-lowering drug, 19 had empagliflozin, 16 had linagliptin, and 20 had baricitinib (Figure 2). When assessing the individual best drug effect compared to the effect of each separate other drug for every individual, results remain similar, with differences ranging from −21.2% (95% CI −25.5, −16.8%; $P < 0.001$) compared to telmisartan treatment to −16.2% (95% CI −21.1, −11.2%; $P < 0.001$) compared to baricitinib (Table 2). Stratifying by diabetes type shows generally smaller changes in suPAR for type 1 diabetes participants, but the individual best drug versus the other three combined, and separately, showed in all cases a significant difference in suPAR levels ranging −12.3% to −24.9%, all $P < 0.001$ (Table S3, Figure S2). A sensitivity analysis of the best individual suPAR change versus the three other drugs, further adjusted for suPAR level at the start of each period, yielded similar results and significance (best vs. others difference: −16.1% [95% CI −19.1, −13.1%]; $P < 0.001$).

3.3 | Associations between suPAR and UACR

Results on the UACR-lowering effect in the ROTATE trials have been published.[13] In short, valid UACR measurements were available for 63 participants and the median (IQR) UACR at baseline was 115 (66–285) mg/g. The individuals' best-performing drug changed UACR from baseline by a mean of −39.6% (95% CI −44.8, −33.8%; $P < 0.001$). Telmisartan was the best-performing UACR-lowering drug for 33 participants (52%), empagliflozin and linagliptin for 11 (17%) and baricitinib for eight participants (13%).

We assessed baseline suPAR level in association with the odds of one specific drug being the individual best UACR-lowering treatment. These analyses showed no associations between baseline suPAR levels and higher odds of a specific drug being identified as more effective in reducing UACR (Table S4).

Correlation analysis of baseline suPAR and UACR levels showed significant correlation in the total population ($R = 0.28$, $P = 0.029$)

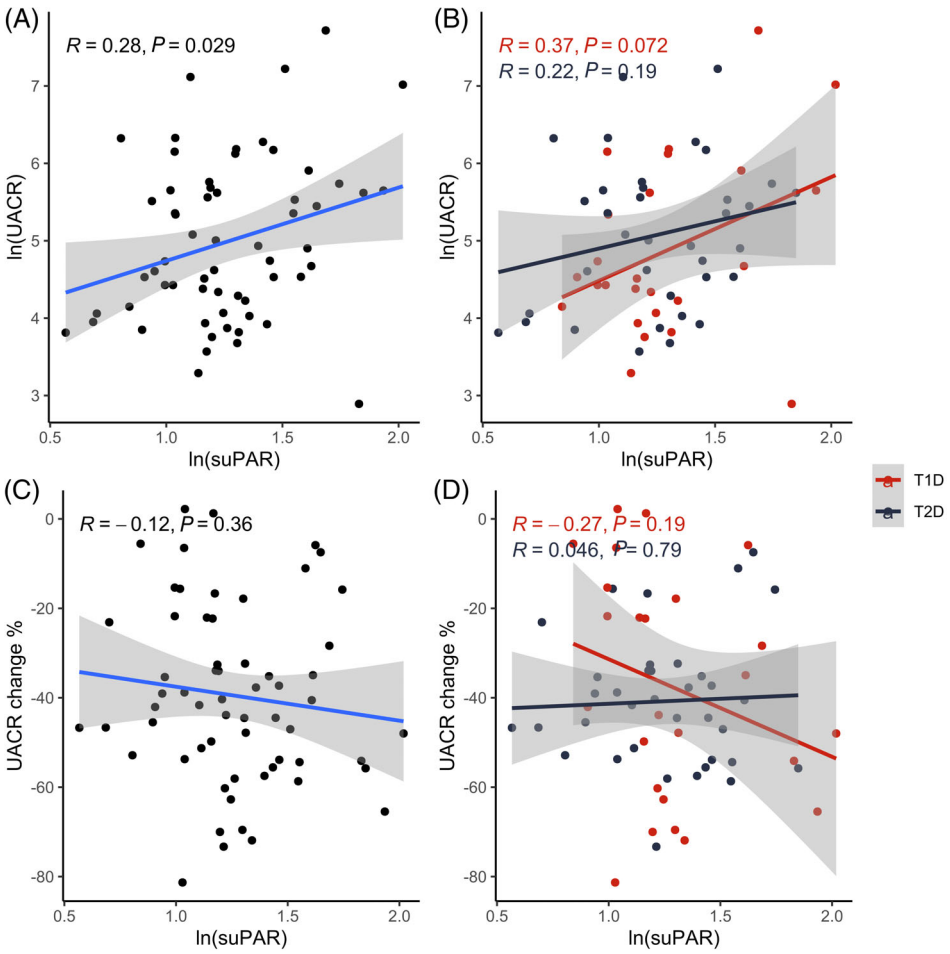

FIGURE 3 Correlation plots assessing first the association between baseline soluble urokinase plasminogen activator receptor (suPAR) and baseline urinary albumin-creatinine ratio (UACR) for the overall population (A) and stratified by diabetes type (B) and second, the association between baseline suPAR levels and the individual best UACR-lowering drug response in the overall population (C) and stratified by diabetes type (D). T1D, type 1 diabetes; T2D, type 2 diabetes

and similar correlation estimates when stratified by diabetes type although these were not significant (type 1 diabetes: 0.37, $P = 0.072$; type 2 diabetes: 0.22, $P = 0.19$ [Figure 3A,B]). When assessing baseline suPAR level with the individual best UACR-lowering drug response, no significant correlation could be identified, either in the overall population or stratified by diabetes type (Figure 3C,D). Further correlation analysis of change in suPAR and change in UACR after treatment with the individual best suPAR-lowering drug likewise showed no significant correlation in the overall, diabetes type-stratified or drug-stratified analyses (Figure S3). Change in suPAR and change in UACR after treatment with the individual best UACR-lowering drug showed no significance in the overall population, nor in the drug-stratified analysis, but a significant negative correlation in type 1 diabetes participants could be seen, as higher UACR decline was correlated with higher suPAR increase ($R = -0.40$, $P = 0.045$ [Figure S4]).

4 | DISCUSSION

In this post hoc analysis of the ROTATE trials, we observed a large variation in the suPAR-lowering response in people with type 1 and type 2 diabetes alike. No single drug evaluated in this study lowered average suPAR significantly, globally, for the total population; however, selecting the individual best-performing drug for each participant showed an overall 13.3% reduction in suPAR levels and a 19.7% reduction in suPAR levels compared to the other three drugs. The individual best-performing drug also varied, with baricitinib being almost twice as likely to be the best-performing drug compared to telmisartan. Furthermore, almost all participants with type 1 diabetes responded best when receiving either baricitinib or empagliflozin, while the best drug for participants with type 2 diabetes varied across all four drugs. Thus, we showed that individual selection of one of the drug classes may lead to a significant reduction of plasma suPAR levels, when blanket treatment with one drug class does not. Furthermore, we showed that the effect these drugs may have on plasma suPAR levels is disassociated from the effect the drugs have on UACR, despite correlation between the two markers at baseline.

suPAR is the soluble version of urokinase plasminogen activator receptor, a marker that is highly active in a multitude of inflammatory processes.[15] suPAR has been demonstrated to be a risk marker of chronic and acute kidney disease, kidney function decline and diabetic complications, and of mortality and hospitalization duration in emergency department settings.[16–21] In this study, individuals with baseline suPAR levels above the median had a significantly lower eGFR compared to those below the median; likely due both to a more

severe disease state and to renal excretion of suPAR potentially leading to falsely elevated suPAR levels with lower eGFR. Evidence concerning disease causality of suPAR has primarily been limited to in vitro and mouse models,[22,23] but a recent combined meta-analysis, genome-wide association study, and Mendelian randomization study in humans indicated a causal role of suPAR in atherosclerosis through monocyte modulation.[7] While similar studies are warranted before causality can be determined in other diseases and complications, these results reveal the need to further explore the role of suPAR as not only a risk marker, but a treatment target as well. We have previously published a study in which the effect of dapagliflozin, an SGLT2 inhibitor, on plasma suPAR levels 12 hours after intake, and after 12 weeks of treatment, was investigated, but no overall effect on suPAR was demonstrated.[8] Another previous study investigating the effect of 8 weeks of treatment with renin-angiotensin system blockade on urinary suPAR levels, however, showed a significant decrease compared to placebo in people with type 2 diabetes.[24]

The drug classes investigated in this trial have large differences in their mode of action and in the tissues in which they are active—although complete characterizations of their effects are still lacking. The RAAS and its inhibition are primarily located in the kidney glomerulus and, although anti-inflammatory effects of RAAS inhibitors have been demonstrated, they are mostly hypothesized to indirectly lower inflammatory responses through an effect on homeostasis,[25] although direct effects through the angiotensin II type I receptor axis have also been reported.[26] SGLT2 is a transporter mainly present in the proximal tubules of the kidneys. Inhibition of SGLT2 has been shown to affect various homeostatic and inflammatory processes, but the specific processes leading to the reno- and cardioprotective benefits of SGLT2 inhibition have not been fully elucidated, although several potential mechanisms exist.[27] In turn, dipeptidyl peptidase-4 is present in the blood and is highly involved in adipose inflammation, promoting hyperinsulinaemia and insulin resistance.[28,29] Finally, Janus kinases, along with signal transducers and activators of transcription are ubiquitous in the extra- to intracellular signalling pathways of cytokines and growth factors, making Janus kinase inhibition a relevant target for a myriad of inflammatory diseases.[30] Thus, the individualized change in suPAR levels of these drugs may be attributable to changes in linked factors, all affecting a generalized inflammatory response in charge of suPAR activation. However, in order to investigate these relationships sufficiently, specific mechanistic studies are needed.

Limitations of the present study include its sample size and design. In this post hoc analysis, we investigated the treatment effect of suPAR in a total of 66 participants, and also subdivided the cohort into diabetes types and treatment categories in some analyses. Both the sample size and the inherent heterogeneity between type 1 and type 2 diabetes limits the applicability of our results. Furthermore, the original trial was designed to assess individual responses with the investigated drugs with regard to UACR levels, and a confirmation period was included to validate the responses during the first exposure to the best individual drug. In this post hoc analysis, we could not include a confirmatory period, which limits the interpretation of our results and increases the risk of a type I error. However, the confirmation period UACR response was significantly correlated with the initial best UACR-lowering response (Pearson $R = 0.39$, 95% CI 0.16–0.66; $P = 0.017$).[13] Although we could not test this, we speculate that similar results would be seen if a confirmatory analysis of suPAR response had been carried out. Interestingly, if the suPAR-lowering effect is indeed valid, we have shown that there is no correlation with the corresponding UACR response, indicating potentially different intra-individual pathophysiological mechanisms activated by the investigated drugs. Similarly, because the study was designed using individuals as their own controls, we lacked an active versus placebo treatment comparison, and as the study was designed to assess the single-drug effect of several guideline-recommended drugs used in multifactorial diabetes treatment, the participants did not adhere to standard of care, thereby inhibiting the generalizability of our results. Furthermore, treatment over 4 weeks might be too short a period to properly assess treatment effect on suPAR. Our results should therefore be considered as hypothesis-generating. Nevertheless, a clear strength of the unique rotational crossover design is that we were able to isolate the specific individual effects of each drug in a personalized manner similar to actual clinical treatment strategies.

To summarize, we found no overall drug effect on suPAR for any of the four investigated drugs. However, we have shown that an individualized treatment strategy across several drug classes may potentially reduce circulating suPAR levels in people with type 1 or type 2 diabetes. Furthermore, the drugs' effects on suPAR are disassociated from their effect on UACR, implying the need for more nuanced treatment selection and for further elucidation of the pathophysiology behind diabetic complications.

AUTHOR CONTRIBUTIONS

Viktor Rotbain Curovic wrote the first draft and performed the statistical analyses. Viktor Rotbain Curovic, Hiddo Heerspink, Frederik Persson and Peter Rossing designed the study. Viktor Rotbain Curovic, Morten Houlind, Marjolein Kroonen, Emilie Zobel, Tine Hansen, Juliette Tavenier, Jesper Eugen-Olsen, Gozewijn Laverman and Adriaan Kooy were involved in data collection and interpretation. All authors revised the draft manuscript. All authors approved the submission for publication.

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CONFLICT OF INTEREST STATEMENT

Viktor Rotbain Curovic, Morten Houlind, Marjolein Kroonen, Tine Hansen, Juliette Tavenier and Adriaan Kooy have no competing

interests to declare. Niels Jongs has received financial support from AstraZeneca to attend meetings/congresses. Emilie Zobel is a full-time employee of Novo Nordisk A/S. Jesper Eugen-Olsen is CSO, cofounder and shareholder in ViroGates, and is named inventor on patents on suPAR, owned by Copenhagen University Hospital Hvidovre, Denmark. Gozewijn Laverman has received lecture fees from Sanofi, AstraZeneca and Janssen, and has served as a consultant for Abbvie, Sanofi, Novo Nordisk, AstraZeneca, Boehringer Ingelheim and Merck Sharp & Dohme. Adriaan Kooy has received research grants from Boehringer Ingelheim and Novo Nordisk. Frederik Persson has served as a consultant, on advisory boards or as educator for AstraZeneca, Novo Nordisk, Boehringer Ingelheim, Sanofi, Mundipharma, MSD, Novartis and Amgen, and has received research grants to his institution from Novo Nordisk, Boehringer Ingelheim, Amgen and AstraZeneca. Peter Rossing has received consultancy and/or speaking fees (to his institution) from Astellas, AstraZeneca, Bayer, Boehringer Ingelheim, Eli Lilly, Gilead, MSD, Mundipharma, Novo Nordisk, Vifor and Sanofi Aventis, and holds research grants from AstraZeneca and Novo Nordisk. Hiddo Heerspink is a consultant for AstraZeneca, Bayer, Boehringer Ingelheim, Chinook, CSL Behring, Eli-Lilly, Gilead, GoldFinch, Janssen, Merck, Mundi Pharma, Mitsubishi Tanabe, Novo Nordisk and Travere Pharmaceuticals, and has received research support from Abbvie, AstraZeneca, Boehringer Ingelheim, Janssen and Novo Nordisk.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.15209.

DATA AVAILABILITY STATEMENT

De-identified participant data will be made available on reasonable request 2 years after the date of publication. Requests should be directed to the senior author (Hiddo J. L. Heerspink). Those submitting a request will be required to send a protocol, statistical analysis plan, and sign a data access agreement to ensure the appropriate use of the study data.

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REFERENCES

1. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney Int*. 2011;80(12):1258-1270.
2. Bikbov B, Purcell CA, Levey AS, et al. Global, regional, and national burden of chronic kidney disease, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2020;395(10225):709-733.
3. Jankowski J, Floege J, Fliser D, Böhm M, Marx N. Cardiovascular disease in chronic kidney disease: pathophysiological insights and therapeutic options. *Circulation*. 2021;143(11):1157-1172.
4. Perkovic V, Jardine MJ, Neal B, et al. Canagliflozin and renal outcomes in type 2 diabetes and nephropathy. *N Engl J Med*. 2019;380(24):2295-2306.
5. Heerspink HJL, Stefánsson BV, Correa-Rotter R, et al. Dapagliflozin in patients with chronic kidney disease. *N Engl J Med*. 2020;383(15):1436-1446.
6. Bakris GL, Agarwal R, Anker SD, et al. Effect of finerenone on chronic kidney disease outcomes in type 2 diabetes. *N Engl J Med*. 2020;383(23):2219-2229.
7. Hindy G, Tyrrell DJ, Vasbinder A, et al. Increased soluble urokinase plasminogen activator levels modulate monocyte function to promote atherosclerosis. *J Clin Invest*. 2022;132(24):e158788.
8. Rotbain Curovic V, Houlind MB, Hansen TW, et al. Acute and long-term treatment with dapagliflozin and association with serum soluble urokinase plasminogen activator receptor. *Front Pharmacol*. 2022;13:13.
9. Heerspink HJL, Perco P, Mulder S, et al. Canagliflozin reduces inflammation and fibrosis biomarkers: a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. *Diabetologia*. 2019;62(7):1154-1166.
10. Packer M. Mechanisms leading to differential hypoxia-inducible factor signaling in the diabetic kidney: modulation by SGLT2 inhibitors and hypoxia mimetics. *Am J Kidney Dis*. 2021;77(2):280-286.
11. Eijkelkamp WBA, Zhang Z, Remuzzi G, et al. Albuminuria is a target for renoprotective therapy independent from blood pressure in patients with type 2 diabetic nephropathy: post hoc analysis from the reduction of endpoints in NIDDM with the angiotensin II antagonist losartan (RENAAL) trial. *J Am Soc Nephrol*. 2007;18(5):1540-1546.
12. Laverman GD, de Zeeuw D, Navis G. Between-patient differences in the renal response to renin-angiotensin system intervention: clue to optimising renoprotective therapy? *J Renin Angiotensin Aldosterone Syst*. 2002;3(4):205-213.
13. Curovic VR, Jongs N, Kroonen MYAM, et al. Optimization of albuminuria-lowering treatment in diabetes by crossover rotation to four different drug classes: a randomized crossover trial. *Diabetes Care*. 2023;46:593-601. doi:10.2337/dc22-1699
14. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.
15. Thunø M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. *Dis Markers*. 2009;27(3):157-172.
16. Hayek SS, Sever S, Ko Y-A, et al. Soluble urokinase receptor and chronic kidney disease. *N Engl J Med*. 2015;373(20):1916-1925.
17. Hayek SS, Leaf DE, Samman Tahhan A, et al. Soluble urokinase receptor and acute kidney injury. *N Engl J Med*. 2020;382(5):416-426.
18. Curovic VR, Theilade S, Winther SA, et al. Soluble urokinase plasminogen activator receptor predicts cardiovascular events, kidney function decline, and mortality in patients with type 1 diabetes. *Diabetes Care*. 2019;42(6):1112-1119.
19. Schultz M, Rasmussen LJH, Andersen MH, et al. Use of the prognostic biomarker suPAR in the emergency department improves risk stratification but has no effect on mortality: a cluster-randomized clinical trial (TRIAGE III). *Scand J Trauma Resusc Emerg Med*. 2018;26(1):69.
20. Iversen E, Kallemose T, Hornum M, et al. Soluble urokinase plasminogen activator receptor and decline in kidney function among patients without kidney disease. *Clin Kidney J*. 2022;15(8):1534-1541.
21. Iversen E, Houlind MB, Kallemose T, et al. Elevated suPAR is an independent risk marker for incident kidney disease in acute medical patients. *Front Cell Dev Biol*. 2020;8:339.
22. Wei C, El Hindi S, Li J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*. 2011;17(8):952-960.
23. Wei C, Möller CC, Altintas MM, et al. Modification of kidney barrier function by the urokinase receptor. *Nat Med*. 2008;14(1):55-63.
24. Persson F, Theilade S, Eugen-Olsen J, Rossing P, Parving H-H. Renin angiotensin system blockade reduces urinary levels of soluble

urokinase plasminogen activator receptor (suPAR) in patients with type 2 diabetes. *J Diabetes Complications*. 2016;30(8):1440-1442.
25. Ekholm M, Kahan T. The impact of the renin-angiotensin-aldosterone system on inflammation, coagulation, and atherothrombotic complications, and to aggravated COVID-19. *Front Pharmacol*. 2021;12:640185.
26. Yisireyili M, Uchida Y, Yamamoto K, et al. Angiotensin receptor blocker irbesartan reduces stress-induced intestinal inflammation via AT1a signaling and ACE2-dependent mechanism in mice. *Brain Behav Immun*. 2018;69:167-179.
27. Cowie MR, Fisher M. SGLT2 inhibitors: mechanisms of cardiovascular benefit beyond glycaemic control. *Nat Rev Cardiol*. 2020;17(12):761-772.
28. Ghorpade DS, Ozcan L, Zheng Z, et al. Hepatocyte-secreted DPP4 in obesity promotes adipose inflammation and insulin resistance. *Nature*. 2018;555(7698):673-677.
29. Love KM, Liu Z. DPP4 activity, hyperinsulinemia, and atherosclerosis. *J Clin Endocrinol Metab*. 2021;106(6):1553-1565.
30. Tanaka Y, Luo Y, O'Shea JJ, Nakayamada S. Janus kinase-targeting therapies in rheumatology: a mechanisms-based approach. *Nat Rev Rheumatol*. 2022;18(3):133-145.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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