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Early Biomarkers of Diabetic Kidney Disease. A focus on albuminuria and a new combination of antidiabetic agents

Running Title: Albuminuria and new antidiabetic drugs.

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Abstract:

Aims: We aimed to determine the efficacy and safety of sodium-glucose cotransporter 2 inhibitors (SGLT2i) and glucagon-like peptide-1 receptor agonists *(*GLP-1ra) to prevent worsening urinary albumin-to-creatinine ratio as an early biomarker of diabetes kidney disease.

Methods: A total of 178 patients with type 2 diabetes and obesity received combination treatment with SGLT2i added to GLP1ra (n=76), GLP1ra added to SGLT2i (n=50) or GLP1ra plus SGLT2i from start (n=52), according to investigators´ best clinical judgment. Major outcomes assessed at 26 weeks were changes in urinary-albumin creatinine ratio (UACR), estimated glomerular filtration rate (eGFR), glycated hemoglobin, body weight, and systolic blood pressure.
Results: All patients (58.6% men, mean age 61.9±10.0years) completed the study. Baseline HbA1c, weight and eGFR levels were 8.2±0.9%, 109.9±19kg and 83.3±19.6 mL/min/m^2, respectively. At 26 weeks, we found significant reductions in HbA1c (1.16%), weight (5.17kg), and systolic blood pressure (8.13mmHg). The reduction in UACR was [15.14 mg/g (95%CI 8.50-22.4)] (-24.6+64.7%); that was greatest in the group of patients with SGLT2i added on to GLP1ra therapy (116.7 mg/g; 95% CI: 54-296.5 mg/g; P<.001. Patients with urinary albumin-to-creatinine ratio > 30 mg/g, showed a higher declines [63.18 mg/g (95%CI 44.5-104.99)] (-56+65.9%). The greatest reduction in urinary albumin-to-creatinine ratio was obtained when SGLT2i was added to GLP1ra (116.7 mg/g). The eGFR did not significantly change along the study period
Conclusion: Our results show the beneficial effect of GLP1ra and SGLT2i combination therapy on early biomarkers of diabetes kidney disease such as albuminuria, and in other significant outcomes for diabetes control.

Keywords: GLP-1ra, SGLT2i, albuminuria, microalbuminuria, diabetic kidney disease.

What's Known?

1. Diabetic kidney disease is highly prevalent.
2. Changes in albuminuria reflect diabetic kidney disease itself.
3. Few therapies are available and thus, there is a substantial unmet clinical need.

4. There is little evidence on both drugs' effect on DKD when used in combination.

What's New?

1. Albuminuria, quantified by the urine albumin-to-creatinine ratio (UACR), could be an easy-to-use surrogate endpoint for choosing the best early treatment option for diabetic kidney disease.
2. For many patients, particularly those identified in early stages of the disease, detection and intervention are essential to improving outcomes in DKD.
3. Use of specific medication such as SGLT2i or GLP-1ra in those with increased albuminuria should be considered. Our results show the beneficial effect of the combination of GLP-1ra plus SGLT2i on DKD, especially on albuminuria.
4. From the patient's perspective, in terms of possible clinical implications, this means that many patients returned to normal or minimally elevated levels of albuminuria (A1 KDIGO categories).

Introduction

Type 2 diabetes mellitus (T2DM) is the main cause of chronic kidney disease and end-stage renal disease. The pathophysiology of diabetic kidney disease (DKD) in patients with type 2 diabetes is complex, especially due to the involvement of other comorbidities such as hypertension, obesity, and dyslipidemia, all of which are associated with insulin resistance, inflammation, and oxidative stress. Furthermore, approximately 35%-75% of diabetes complications are estimated to be attributed to hypertension[1].

DKD is functionally characterized by early glomerular hyperfiltration and increased albuminuria followed by a progressive decline in the estimated glomerular filtration rate (eGFR) until end-stage renal disease is developed[2]. In patients with type 2 diabetes, the mean annual incidence of microalbuminuria is estimated to be 8%, the incidence of developing eGFR <60 ml/min/1.73m^2 (according to the Modification of Diet on Renal Disease equation) is estimated to be 2–4%, and the incidence of end-stage renal disease is estimated to be 0.04% to 1.8%[3].

Despite important advances in type 2 diabetes management, DKD continues to lead to dramatically shortened lifespans and reduced quality of life in addition to increased healthcare

costs[4]. Advances in knowledge on its the pathophysiology and increased eligibility for treatment of end-stage renal disease entail a need for better prediction, prevention, and treatment[5]. Tight glycemic control and optimal blood pressure control have been mainstays for preventing microvascular outcomes. However, long-term follow-up is required in order to improve renal outcomes, especially when chronic kidney disease is already present[6].

The onset and progression of albuminuria is an early biomarker of renal microangiopathy and a predictor of DKD[7]. According to the biological underpinnings, it is highly plausible that changes in albuminuria could be a surrogate endpoint for progression of DKD[8]. New glucose-lowering agents, especially glucagon-like peptide-1 receptor agonists (GLP-1ra) and sodium-glucose cotransporter type 2 inhibitors (SGLT2i), as add-on therapies have been shown to have beneficial positive effects on composite renal outcomes in T2DM patients with or without established cardiovascular disease[9]. SGLT2i is associated with a reduction in glomerular hyperfiltration, mediated through increased natriuresis and restored tubuloglomerular feedback. This may improve renal oxygenation and cellular energy metabolism as well as reduce intrarenal inflammation. In addition, they may have additional anti-inflammatory and antifibrotic effects. The main effect of GLP-1ra is driven by a reduction in albuminuria. GLP-1 is expressed in both preglomerular and juxtaglomerular smooth muscle cells of the vessels. Through a reduction in sodium–hydrogen exchanger 3, natriuresis and osmotic diuresis increase. It reduces resistance of the afferent arteriole and increases resistance of the efferent arteriole, either through the GLP-1 receptor, nitric oxide, or other vascular factors. Therefore, it has an antifibrotic and anti-inflammatory effect[2].

These newer glucose-lowering approaches seem to be promising for the prevention and treatment of DKD in patients with type 2 diabetes, with effects that go beyond improved glycemic control[10,11].

Aim

The principal aim of this study was to determine the association between the effects of predetermined treatment schedules on early changes in albuminuria and to provide information on the use of albuminuria as a surrogate endpoint useful for choosing the best treatment option.

We hypothesize that, first, albuminuria, considered easy-to-use biomarker of DKD, could allow clinicians to adopt effective measures that must be taken in order to avoid disease progression and perhaps help in selecting the most efficient treatment options. Secondly, we hypothesize that the combined use of antidiabetic drugs, mainly GLP-1ra and SGLT2i, will have a positive, reinforced effect on DKD progression measured via a decrease in albuminuria, as has been shown with each drug individually.

Materials and Methods

We conducted an observational, prospective, multicenter study based on clinical practice. The aim was to assess the efficacy of the combined use of SGLT2i and GLP-1ra on renal outcomes, as measured by the percentage decrease in the urine albumin-to-creatinine ratio (UACR) at 26 weeks in outpatients attended to by Internal Medicine Departments.

Patients

Patients with albuminuria as determined by the urine albumin-to-creatinine ratio who received combination therapy with GLP-1ra and SGLT2i were included. The exclusion criteria were type 1 diabetes mellitus, a medical history of diabetic ketoacidosis, complex insulin therapies (more than two daily doses), eGFR <45 mL/min/m^2, and a medical history of medullary thyroid cancer or pancreatitis. Patients were followed-up on for 26 weeks and all events were assessed by the centers' investigators.

Data were collected in an anonymized electronic registry. This work was conducted in accordance with the ethical principles for research on human beings outlined in the Declaration of Helsinki and updated at the General Assembly of Brazil (2013). The confidentiality and secrecy of personal data were respected. This study was approved by the Clinical Research Ethics Committee of the University Hospital of Badajoz. Patients signed an informed consent form available on the registry web site.

Variables

Clinical and anthropometric variables were obtained from all participating patients. eGFRs were calculated using the Chronic Kidney Disease Epidemiology Collaboration equation[12].

Albuminuria was quantified using UACR. Stages of albuminuria were defined as follows: A1 (UACR <30 mg/g), A2 (UACR 30-300 mg/g), and A3 (UACR >300 mg/g)[13].

Patients were stratified into 3 groups according to sequential order of treatment. In group one, GLP-1ra was started first and SGLT2i second. In group two, SGLT2i was started first and GLP-1ra second. In group three, both drugs were started simultaneously

Endpoints

The primary endpoint was mean change (SD) in UACR levels at 26 weeks in the entire cohort and in each of the three treatment schedule groups. The secondary endpoints were changes in glycated hemoglobin levels, body weight, systolic blood pressure, and triglyceride levels at 26 weeks.

Statistical analysis

Mean and standard deviation or median and interquartile range were used depending on normality of the variables. Qualitative variables are expressed as absolute numbers (percentages). In order to compare qualitative variables among subgroups, we used the chi-square test. In order to compare quantitative variables among subgroups, we used the ANOVA test in the case of normal distribution, the Welch's t-test in the case of unequal variance, and the Kruskal–Wallis test in the case of non-normal data. To compare results during the follow-up period, we used the paired t-test when normality and homoscedasticity were proven and the Wilcoxon signed-rank test for the remaining variables. Differences were expressed as mean/median and 95% confidence interval. All statistical analyses were performed using R, v. 3.3.2. $p<0.05$ was considered statistically significant.

In order to detect a 30% change in urine albumin-to-creatinine ratio after treatment with a standard deviation of 1, a confidence level of 90%, and a statistical power of 90%, a sample of more than 119 patients was required[8].

Results

The study included 178 patients (58.6% males). Patients' mean age was 61.9±10.0 years. Patients had had type 2 diabetes for a mean of 9.2±5.9 years. Mean body mass index was 36.2±10 kg/m^2. Baseline glycated hemoglobin and eGFR levels were 8.2±0.9% (66 mm/mol) and 83.3±19.6 mL/min/m^2, respectively. In terms of comorbidities, 80.9% of patients had a history of hypertension, 81.4% of dyslipidemia, and 7.9% of coronary artery disease. Clinical and anthropometric variables at baseline according to the predetermined treatment schedule group are shown in Table 1.

The most commonly used GLP-1ra was liraglutide (52.2%) followed by dulaglutide (33.1%), exenatide LAR (7.3%), lixisenatide (4.5%), and albiglutide (2.8%). The most commonly used SGLT2i was canagliflozin (46.6%) followed by dapagliflozin (29.8%) and empagliflozin (23.6%). Of the total sample, 28.1% of patients were taking angiotensin-converting enzyme inhibitor(s), 42.7% were taking an angiotensin II receptor blocker, 26.4% were taking thiazide diuretics, and 22.5% were taking loop diuretics, with no significant differences among the prespecified subgroups (Table 1).

Follow-up at 26 weeks

The main findings upon follow-up are shown in Table 2. We found a significant reduction in glycated hemoglobin [-1.16% (95%CI 0.97-1.35) p<0.0001], weight [-5.17 Kg (95%CI 0.97-1.35) p<0.0001], triglyceride levels [-16 mg/dl (95%CI 3.49-28.99) p<0.05], and systolic blood pressure [-8.13 mmHg (95%CI 5.77-10.48) p<0.0001], among other factors.

Results by Urine Albumin-To-Creatinine ratio subgroups

Table 2 shows findings at 26 weeks according to baseline UACR subgroup. We found a significant reduction in UACR in all patients regardless of baseline levels [-15.14 mg/g (95% CI 8.50-22.49) p<0.0001] at 26 weeks after beginning treatment. The percentage change was 24.6+64.7%, with no differences found among groups. 77 patients had a decrease of greater than 30%. Patients who were in KDIGO[13] (Kidney Disease Improving Global Outcomes) categories A2 and A3 (UACR >30 mg/g) (n=69) showed greater declines [-63.184 mg/g (95% CI 44.5-104.99) p<0.0001], with a mean improvement of 56+65.9%.

Results by treatment schedule subgroup

The total sample was classified into groups according to whether patients began combination therapy with GLP-1ra, with SGLT2i, or started both agents simultaneously. The principal findings are shown in Table 2. Regarding glycated hemoglobin, weight, and systolic blood pressure, the greatest reductions at 26 weeks were seen in patients who started a combination of both drugs simultaneously ([-1.69% (95%CI 1.22-2.16) p<0.0001], [-7.6 Kg (95%CI 4.13-11.18) p<0.0001], [-9.96 mmHg (95%CI 4.92-15.01) p<0.0001] respectively). No significant changes were seen in eGFR between groups.

In terms of UACR, at 26 weeks we found a significant decline in mean total UACR and macroalbuminuria (UACR >30 mg/g) ([-15.14 mg/g (95%CI 8.50-22.49) p<0.0001] and [-63.18 mg/g (44.5-104.99) p<0.0001]). Similar changes in total UACR were observed when both drugs were started at the same time and when GLP-1ra was added to SGLT2i (-17.17 mg/g and -16.4 mg/g respectively; p<0.0001). On the contrary, in terms of macroalbuminuria (n=69), a greater change was observed when SGLT2i was added to GLP-1ra (-116.7 mg/g; p<0.005). As shown in Figure 1, 38.8% of patients were in A2 or A3 KDIGO categories at baseline. At 26 weeks, this proportion fell to 24.2%. From the patient's perspective and in terms of possible clinical implications, this means that 14.6% returned to normal or minimally elevated levels of albuminuria (A1 KDIGO).

Discussion

In clinical practice, DKD is diagnosed by albuminuria, a decrease in estimated eGFR, or both[14]. Albuminuria is primarily a measure of the permeability of the glomerular capillary wall by macromolecules[15]. Increases in urine albumin to microalbuminuria or macroalbuminuria levels are not only strongly associated with progression to ESRD, but also with an increased risk of cardiovascular complications[16,17]. In cohorts and clinical trials, an average UACR reduction of 20-30% over a 2-year interval is associated with a hazard ratio (HR) of 0.7 for clinical outcomes [17].

Our study found an early beneficial effect of GLP-1ra and SGLT2i combination therapy at 26 weeks. Specifically, we observed a beneficial reduction in UACR (24.6+64.7%), with larger

reductions in patients with baseline UACR levels >30 mg/g (56+65.9%). The eGFR did not significantly change along the study period. A recent meta-analysis found that a 25% reduction in UACR is necessary to observe clinical benefits. Furthermore, the meta-analysis found that in participants with UACR levels >30 mg/g, a 20% decrease in UACR would be required[17]. For many patients, especially those who are identified in early stages of the disease, early detection and intervention are essential for improving DKD outcomes and indeed, changes in albuminuria reflect the disease itself. Regarding safety concerns, all patients who participated in our study finished the study period and there were no adverse events leading to drug discontinuation.

The beneficial effect of SGLT2i on renal outcomes has been consistently demonstrated in large randomized clinical trials, with 7,000 to 17,000 patients recruited per trial. Empagliflozin, canagliflozin, and dapagliflozin characteristically reduced the annual decline in eGFR and mean UACR when compared with a placebo[18-20]. Regarding GLP-1ra, in a prespecified secondary analysis, treatment with liraglutide, semaglutide, and dulaglutide had a significantly lower rates of new or persistent macroalbuminuria[21-23].

There are few clinical trials that report the renal effects of the combination of GLP-1ra and SGLT2i. Most trials have evaluated the effect of GLP-1ra as an add-on therapy to SGLT2i, with study sample sizes of between 300 and 400 patients[24-26]. As expected, trials have found that dulaglutide, semaglutide, and liraglutide as add-on therapies to SGLT2i lead to significant reductions in serum glucose concentrations, glycated hemoglobin, and body weight when compared to a placebo. However, the effect of combined GLP-1ra and SGLT2i on renal outcomes has not been systematically analyzed as a primary outcome. In two previous trials, neither once-weekly subcutaneous semaglutide as an add-on therapy to SGLT2i nor daily liraglutide added to SGLT2i had a significant effect on UACR or eGFR compared to a placebo at 30 or 26 weeks of follow-up, respectively[25,26]. In both trials, an eGFR <60 ml/min/1.73m^2 was an exclusion criterion. In contrast to these randomized controlled clinical trials, 58% of patients in our study had an eGFR <60 ml/min/1.73m^2 at baseline, meaning they had more advanced disease and a greater chance of obtaining a beneficial effect in such a short observation period.

Moreover, we were able to confirm that combined GLP-1ra and SGLT2i had a greater effect on systolic blood pressure compared to what was found in previous randomized clinical trials. Specifically, we observed a mean reduction of 8.13 mmHg in systolic blood pressure

compared to a 1.43 mmHg and 0.25 mmHg decrease found in randomized clinical trials on semaglutide and liraglutide as add-on therapies to SGLT2i, respectively[25,26]. The effect of GLP-1ra and SGLT2i on lowering systolic blood pressure is an additional factor that explains the benefit of this combination on renal outcomes for patients with DKD[27].

Finally, we would like to remark on the safety of combined GLP-1ra and SGLT2i. We did not observe any severe adverse events leading to drug discontinuation in the observation period. The same safety profile has also been observed in other randomized trials, with drug discontinuation rates due to severe side effects of less than 3%[25,6]. We have also confirmed the safety of GLP-1ra and SGLT2i in a real-world study that includes elderly patients[28].

This study has some limitations. First, as an observational study, it was subject to bias, and the lack of a comparator group does not allow for stronger conclusions to be drawn. Second, this study does not distinguish among different drug doses because there were too few patients in the three groups, even though we managed to exceed the necessary sample size for observing significant benefits.

Conclusions

Diabetic kidney disease is highly prevalent. Few therapies are available and as such, there is a substantial unmet clinical need. Our results show the beneficial effect of the combination of GLP-1ra plus SGLT2i on DKD, especially on early biomarkers such as albuminuria. Indeed, changes in albuminuria reflect changes in DKD itself. For many patients, particularly those who are identified early in their disease, early detection and intervention are essential to improving outcomes in DKD.

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Authorship

All authors made substantial contributions to the conception and design, data acquisition, and analysis and interpretation of data. Carretero Gómez and Arévalo Lorido were involved in drafting the manuscript and revising it critically. All authors gave final approval to the version to be published.

References

1. Epstein M, Sowers JR. Diabetes Mellitus and Hypertension. Hypertension 1992;19:403-18
2. Carretero Gómez J, Arévalo Lorido JC. Clinical assessment and treatment of diabetes in patients with chronic kidney disease. Rev Clin Esp. 2018;218:305-15. doi: 10.1016/j.rce.2018.03.016.

3. Koye DN, Shaw JE, Reid CM, Atkins RC, Reuterns AT, Magliano DJ. Incidence of chronic kidney disease among people with diabetes: a systematic review of observational studies. Diabet Med. 2017;34:887–901
4. Neuen BL, Chadban SJ, Demaio AR, Perkovic V. Chronic kidney disease and the global NCDs agenda**.** BMJ Glob Health. 2017; **2**: e000380. doi: 10.1136/bmjgh-2017-000380
5. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: challenges, progress, and possibilities. Clin J Am Soc Nephrol. 2017;12:2032–2045
6. Ruospo M, Saglimbene VM, Palmer SC, De Cosmo S, Pacilli A, Lamacchia O et al. Glucose targets for preventing diabetic kidney disease and its progression. Cochrane Database Syst Rev. 2017;6:CD010137. DOI:10.1002/14651858.CD010137.pub2.
7. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Chapter 1: Definition and classification of CKD. Kidney Int Suppl. 2013;3:19-62
8. Levey AS, Gansevoort RT, Coresh J, Inker LA, Heerspink HL, Grams ME et al. Change in Albuminuria and GFR as End Points for Clinical Trials in Early Stages of CKD: A Scientific Workshop Sponsored by the National Kidney Foundation in Collaboration With the US Food and Drug Administration and European Medicines Agency. American Journal of Kidney Diseases 2020 75, 84-104. DOI:10.1053/j.ajkd.2019.06.009
9. Zelniker T A, Wiviott S D, Raz I, Im K, Goodrich E L, Furtado R, et al. Comparison of the Comparison of the Effects of Glucagon-Like Peptide Receptor Agonists and Sodium-Glucose Cotransporter 2 Inhibitors for Prevention of Major Adverse Cardiovascular and Renal Outcomes in Type 2 Diabetes Mellitus. Systematic Review and Meta-Analysis of Cardiovascular Outcomes Trials. Circulation. 2019;139:2022–31. DOI: 10.1161/CIRCULATIONAHA.118.038868
10. Neumiller JJ, Alicic RZ, Tuttle KR. Therapeutic considerations for antihyperglycemic agents in diabetic kidney disease. J Am Soc Nephrol 2017;28:2263–74.
11. Goldenberg RM, Berall M, Chan CTM, Cherney DZI, Lovshin JA, McFarlane PA, et al. Managing the course of diabetic kidney disease: from the old to the new. Can J Diabetes 2018;42:325–34

12. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-12
13. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013;3:1-150
14. Parving H-H, Persson F, Rossing P. Microalbuminuria: a parameter that has changed diabetes care. Diabetes Res Clin Pract 2015; 107:1–8
15. Moeller MJ, Tenten V. Renal albumin filtration: alternative models to the standard physical barriers. Nat Rev Nephrol 2013;9:266-77
16. Heerspink HJL, Greene T, Tighiouart H, et al. Change in albuminuria as a surrogate endpoint for progression of kidney disease: a meta- analysis of treatment effects in randomised clinical trials. Lancet Diabetes Endocrinol 2019;7**:**128-39
17. Coresh J, Heerspink HJL, Sang Y, Matsushita K, Arnlov J, Astor BC, et al. Change in albuminuria and subsequent risk of end-stage kidney disease: an individual participant-level consortium meta-analysis of observational studies. Lancet Diabetes Endocrinol. 2019;7:115-27.. DOI: 10.1016/ S2213-8587(18)30314-0.
18. Wanner C, Inzucchi SE, Lachin JM, Fitchett D, von Eynatten M, Mattheus M, et al. Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. N Engl J Med. 2016;375:323-34. doi:10.1056/NEJMoa1515920
19. Perkovic V, de Zeeuw D, Mahaffey KW, Fulcher G, Erondu N, Shaw W, et al. Canagliflozin and renal outcomes in type 2 diabetes: results from the CANVAS Program randomised clinical trials. *Lancet Diabetes Endocrinol.* 2018;6:691-704. doi:10.1016/S2213-8587(18)30141-4
20. Mosenzon O, Wiviott SD, Cahn A, Rozenberg A, Yanuv I, Goodrich E, et al. Effects of dapagliflozin on development and progression of kidney disease in patients with type 2 diabetes: an analysis from the DECLARE-TIMI 58 randomised trial [published correction appears in Lancet Diabetes Endocrinol. 2019 Aug;7:e20]. Lancet Diabetes Endocrinol. 2019;7:606-17. doi:10.1016/S2213-8587(19)30180-9
21. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al. for the LEADER Steering Committee on behalf of the LEADER Trial Investigators.

Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2016;375:311-22.

22. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, et al; SUSTAIN-6 Investigators. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. N Engl J Med 2016;375:1834-44.
23. Gerstein H, Colhoun HM, Dagenais GR, Díaz R, Lakshmanan M, Pais P, et al. Dulaglutide and cardiovascular outcomes in type 2 diabetes (REWIND): a double-blind, randomised placebo-controlled trial. Lancet 2019; 394:121-30. DOI: 10.1016/S0140-6736(19)31149-3
24. Frías JP, Guja C, Hardy E, Ahmed A, Dong F, Öhman P, et al. Exenatide once weekly plus dapagliflozin once daily versus exenatide or dapagliflozin alone in patients with type 2 diabetes inadequately controlled with metformin monotherapy (DURATION-8): A 28-week, multicentre, double-blind, phase 3, randomised controlled trial. Lancet Diabetes Endocrinol 2016;4:1004-16.
25. Zinman B, Bhosekar V, Busch R, Holst I, Ludvik B, Thielke D, et al. Semaglutide once weekly as add-on to SGLT-2 inhibitor therapy in type 2 diabetes (SUSTAIN 9): a randomised, placebo-controlled trial [published correction appears in Lancet Diabetes Endocrinol. 2019 Mar 11;:] [published correction appears in Lancet Diabetes Endocrinol. 2019 Aug;7(8):e20] [published correction appears in Lancet Diabetes Endocrinol. 2019 Nov;7(11):e22]. Lancet Diabetes Endocrinol. 2019;7:356-67. doi:10.1016/S2213-8587(19)30066-X
26. Blonde L, Belousova L, Fainberg U, Garcia-Hernandez PA, Jain SM, Kaltoft MS, et al. Liraglutide as add-on to sodium-glucose co-transporter-2 inhibitors in patients with inadequately controlled type 2 diabetes: LIRA-ADD2SGLT2i, a 26-week, randomized, double-blind, placebo-controlled trial. Diabetes Obes Metab. 2020;22(6):929-937. doi:10.1111/dom.13978
27. Arevalo-Lorido JC, Gómez JC, Huelgas RG, de Lucas DG, Polo LM, Aguilar JMV, et al. Lowering Blood Pressure with the Combination of a Sodium-Glucose Cotransporter 2 Inhibitor and a Glucagon-like Peptide-1 Receptor Agonist in Type 2 Diabetic Patients: A Clinical Evidence. High blood pressure & cardiovascular prevention: the official journal of the Italian Society of Hypertension. 2018;254:417-20.

28. Carretero Gómez J, Arévalo Lorido JC, Gómez Huelgas R, García de Lucas D, Mateos Polo L, Varela Aguilar JM, et al; Diabetes, Obesity, Nutrition Spanish Working Group. Combination Therapy With Glucagon- Like Peptide-1 Receptor Agonists and Sodium- Glucose Cotransporter 2 Inhibitors in Older Patients With Type 2 Diabetes: A Real-World Evidence Study. Can J Diabetes. 2019;43:186-92. DOI: 10.1016/j.jcjd.2018.09.001

Table 1. Clinical and anthropometric variables at baseline by predetermined treatment schedule groups (successive vs. simultaneous treatment)

Variable	Total	SGLT2i added to GLP1ra	GLP1ra added to SGLT2i	GLP1ra plus SGLT2i	p
N	178	76	50	52	
Age	61.9+/-10.0	62.6 +/-9.5	62.7 +/-9.2	60.3 +/- 11.4	0.37
Sex (male)	109 (58.6%)	41 (53.9%)	35 (70%)	28 (53.8%)	0.14
Smoker	32 (17.9%)	11 (14.5%)	11 (22%)	10 (19.2%)	0.5
Dyslipidemia	145 (81.4%)	70 (92.1%)	40 (80%)	35 (67.2%)	0.001
Hypertension	146 (80.9%)	57 (75%)	39 (78%)	48 (92.3%)	0.04
CAD	33 (7.9%)	17 (22.3%)	8 (16%)	7 (13.4%)	0.39
BMI	36.2+/-10	36.6+/-10.5	33.3+/-7	38.1+/-8.1	0.0001
Mean T2DM duration	10+/-6.7	11.1+/-6.2	10.3+/-7.5	8.5+/-6.4	0.11
SBP	138.3+/-16.9	138.1+/-14.6	136.9+/-20.4	140+/-16.7	0.65
DBP	76.8+/-10.8	74.9+/-9.5	77.3+/-11.1	79.3+/-11.9	0.07
HR	75.4+/-10.8	74.6+/-10.7	74.9+/-12.1	77.2+/-9.6	0.4
Hematocrit	41.8+/-5.7	41.5+/-6.2	42.4+/-5.1	41.9+/-5.3	0.67
eGFR	83.3+/-19.6	79.2+/-20.4	84.1+/-18.3	88.4+/-18.0	0.03
LDL-c	88.4+/-35.6	90.6+/-39.4	82.8+/-32	90.6+/-32.7	0.41
HDL-c	42.0+/-15	45.7+/-24.1	47.6+/-21.9	42.7+/-13.8	0.48
Serum Uric Acid	5.3+/-1.7	5.7+/-2	5.1+/-1.5	5.1+/-1.5	0.06
ACEIs	50 (28.1%)	22 (28.9%)	12 (16.1%)	16 (30.7%)	0.87

ARB	76 (42.7%)	28 (36.8%)	25 (54.3%)	23 (44.2%)	0.16
Thiazide	47 (26.4%)	15 (10.7%)	16 (34.8%)	16 (30.8%)	0.14
Loops diuretics	40 (22.5%)	20 (26.3%)	9 (19.6%)	11 (21.1%)	0.16

Data are expressed as mean and SD in case of normality and median and interquartile range for the rest. ACEIs: Angiotensin-converting enzyme inhibitor(s); ARB: Angiotensin II receptor blocker; BMI: body mass index; CAD: coronary artery disease; DBP: diastolic blood pressure; eGFR: glomerular filtration rate; HDL-C: high-density lipoprotein cholesterol; HR: heart rate; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; T2DM: type 2 diabetes mellitus.

Table 2. Predetermined treatment schedule groups (successive vs. simultaneous treatment)

Predetermined groups	Total		SGLT2i added to GLP-1ra		GLP-1ra added to SGLT2i		GLP-1ra plus SGLT2i	
	Baseline	**26 weeks**	**Baseline**	**26 weeks**	**Baseline**	**26 weeks**	**Baseline**	**26 weeks**
N	178		76		50		52	
Weight (Kg)	100.9+/-19	95.1+/-16.7	96.5+/-32.1	93.9+/-24.7	90+/-19.5	87.2+/-16.5	105.5+/-29.6	100.1+/-23
Mean change		-5.17 (3.86-6.47) **		- 3.66 (2.01-5.3) **		-3.92 (2.55-5.30) **		-7.66 (4.13-11.18) **
HbA1c (%)	8.2+/-0.9 (66 mmol /mol)	6.9+/-0.9 (52 mmol/ mol)	7.9+/-0.8 (63 mmol /mol)	7+/-1 (53 mmol/ mol)	8.3+/-0.9 (67 mmol /mol)	6.9+/-1.3 (52 mmol/ mol)	8.5+/-1.1 (69 mmol /mol)	6.8+/-1.1 (51 mmol /mol)
Mean change		-1.16 (0.97-1.35) **		-0.85 (0.59-1.12) **		-1.21 (0.84-1.58) **		-1.69 (1.22-2.16) **
UACR (mg/g)	19.6+/-52.5	10.4+/-22.4	14.8+/-35.5	7.1+/-17.4	25+/-82	12+/-25.4	25.7+/-56.5	17.9+/-29.5
Mean change		-15.14 (8.50-22.49) **		-10 (4.13-38.89) **		-16.4 (8.07-36.3) **		-17.19 (5.35-28.05) **
UACR ≥ 30 (mg/g) (n=69)	95.1+/-222	31.4+/156	127.5+/-415 (n=24)	30+/-202.5	118+/-225.4 (n=21)	45.7+/-154.5	75.34+/-167.9 (n=24)	24.95+/-54.37
Mean change		-63.18		-116.7		-55.5		-41.65

		(44.5-104.99) **		(54.24-296.50) **		(25.85-130.65) **		(30-73.49) **
SBP	138.3+/-16.9	130.4+/-16.8	138.1+/-14.6	130+/-20	136.9+/-20.4	127+/-17.5	140+/-16.7	130+/-19
Mean change		-8.13 (5.77-10.48) **		-8.04 (5.30-10.68) **		-5.8 (0.95-10.64) *		-9.96 (4.92-15.01) **
eGFR (ml/min/1.73m^2)	83.3+/-19.6	82.9+/-23.8	78.3+/-23.9	76.9+/-21.4	85.1+/-19	83.8+/-19	90.8+/-13.5	90.1+/-14.7
Mean change		-1.14 (-1.2-3.4) p=0.32		-1.4 (-2.4 – 5.2) p=0.46		-1.22 (-2.9 – 5.3) p=0.55		-0.6 (-3.5-4.7) p=0.7
Triglycerides (mg/dL)	185.4+/-108.4	166.7+/-91.9	210.7+/-124.1	171.2+/-95.7	171.6+/-91.8	148+/-72	161.8+/-91	138+/-74.5
Mean change		-16 (3.49-28.99) *		-32.50 (9.99-56.99) *		-9.43 (-15.5-33) p=0.5		-5.99 (-11.99-21.49) p=0.5

*p<0.05

**p<0.001

Data are expressed as mean and 95%CI in case of normality (HbA1c, SBP, Weight, eGFR) and median and 95%CI for the rest. eGFR: glomerular filtration rate; HbA1c: glycated hemoglobin; SBP: systolic blood pressure; UACR: urine albumin-to-creatinine ratio

Figure 1. Changes in KDIGO categories between baseline and 26 weeks.

eGFR: glomerular filtration rate.

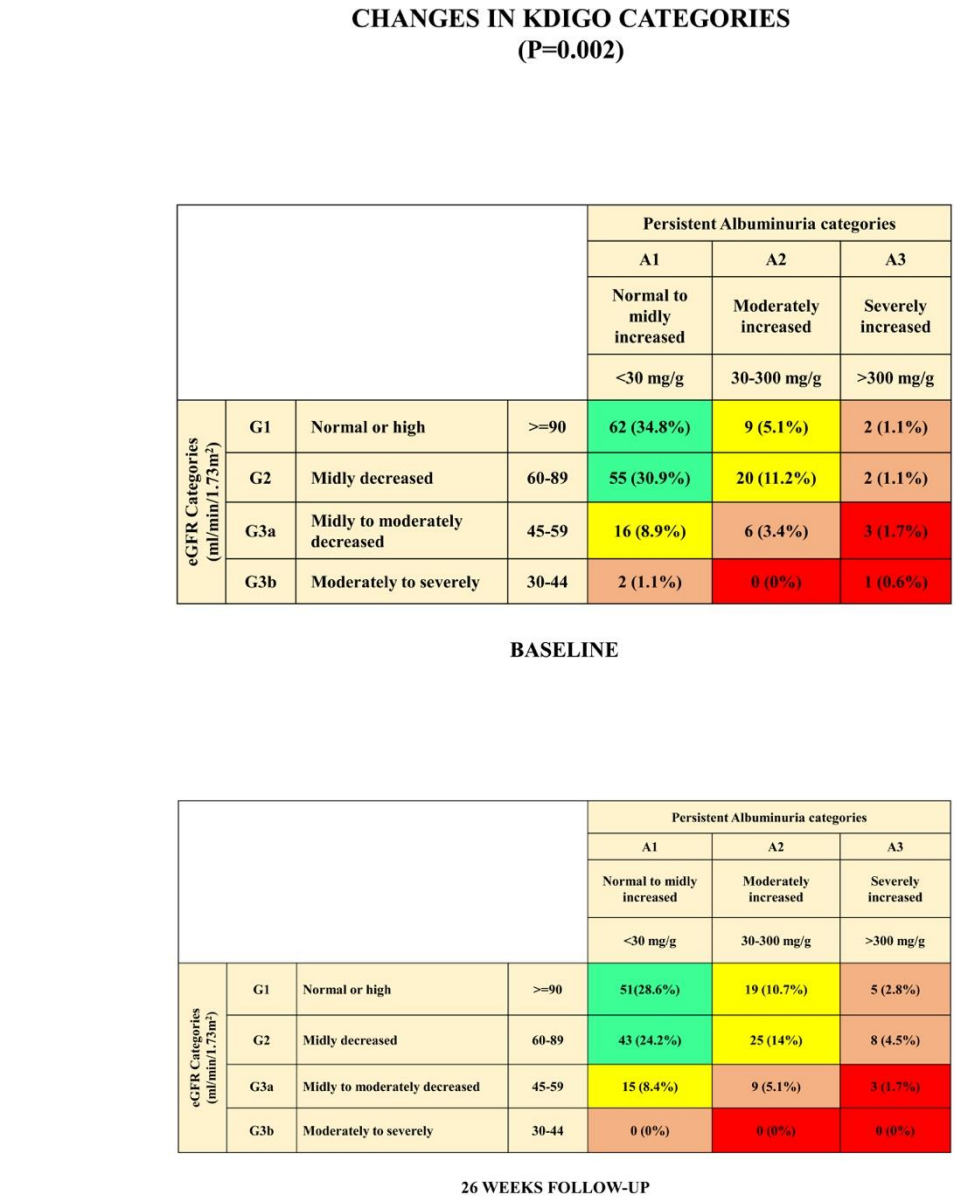

Figure 1. Changes in KDIGO categories at 26 weeks.

eGRF: glomerular filtration rate.