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Tweet: Is higher rate of amputations, believed to be associated with SGLT2 inhibitors, could be related to diuretic effect? Potier et al. found a higher risk of amputations in diuretic users from a French observational study compared with non-users. Figure 1 could be included in the tweet.

Word count: 3001

ABSTRACT

Aims/hypothesis: Recently, safety data signaled an increased risk of amputations in patients taking canagliflizon, a SGLT2 inhibitor. If this side effect is due to drug-induced hypovolemia, diuretics should also increase that risk. The aim of this study was to analyze the association between diuretics use and the risk of lower limb events (LLE) in patients with type 2 diabetes.

Methods: SURDIAGENE is a prospective observational cohort including type 2 diabetes patients enrolled from 2002 to 2012 and followed-up until onset of LLE, death, or December 31, 2015, whichever came first. Primary outcome was the first occurrence of LLE, a composite of lower limb amputations (LLA) and lower limb revascularizations (LLR). Rate of primary outcome was compared between participants taking or not diuretics at baseline in Cox adjusted model.

Results: At baseline, of the 1459 included participants, 670 were taking diuretics (in participants with and without diuretics, mean age was 67.1 and 62.9; 55.8% and 59.8% were men, respectively). During a median follow-up of 7.1 years, the incident of LLE was 1.80 per 100 patient.years in diuretics users versus 1.00 in non-users ($p<0.001$). The hazard ratio for LLE in users vs. non-users was 2.08 [95%CI, 1.49, 2.93; $p<0.001$]. This association remained significant in multivariable adjusted model (1.49 [1.01, 2.19; $p=0.04$]) and similar after considering death as a competing risk (subhazard ratio 1.89 [1.35, 2.64; $p<0.001$]). When separated, LEA but not LLR were associated with the use of diuretics (2.01 [1.14, 3.54; $p=0.02$] and 1.05 [0.67, 1.64; $p=0.84$], respectively in the multivariable adjusted model).

Conclusions: Among patients with type 2 diabetes treated with diuretics, there was a significant increase in the risk of LLE, predominantly LLA.

Key words: diuretics, lower limb amputation, lower limb revascularization, type 2 diabetes

List of abbreviations

ACR: albumin-to-creatinine ratio

CANVAS: Canagliflozin Cardiovascular Assessment Study

LLA: lower limb amputation

LLE: lower limb events

LLR: lower limb revascularization

PAD: peripheral arterial disease

RAAS: renin angiotensin aldosterone system

SGLT2: sodium/glucose cotransporter 2

Research in context

- **What is already known about this subject?**
  - Lower limb amputations and revascularizations are highly prevalent in patients with type 2 diabetes
  - Recently, some evidence from randomized controlled trial and observational data suggested that SGLT2 inhibitors could be associated with an increased risk of lower limb amputations
  - A potential mechanism for the increased risk of lower limb amputations observed with SGLT2 inhibitors could be the glycosuria-induced osmotic diuresis
- **What is the key question?**
  - Is there an association between diuretics use and lower limb events in patients with type 2 diabetes?

- **What are the new findings?**

  - In a single centre prospective observational cohort of patients with type 2 diabetes, diuretics use was associated with a higher risk of lower limb amputations and revascularizations

- **How might this impact on clinical practice in the foreseeable future?**

  - Clinical implications might be to use diuresis-inducing drugs with caution and careful assessment of the volemia in patients with diabetes and peripheral arterial disease or foot ulcers.

BACKGROUND

Diabetes mellitus is the leading cause of lower limb amputations (LLA) [1]. The high prevalence of this debilitating complication in patients with diabetes is explained by a wide range of factors such peripheral arterial disease (PAD), diabetic neuropathy, impaired wound healing, susceptibility to infection and others [2]. Ischemia is associated with a dramatic increase risk of LLA in patients with diabetes and foot ulcer [3]. Hypovolemia could further decrease peripheral perfusion in patients with PAD, favoring decompensation and eventually LLA. There is evidence from case reports that extracellular volume depletion could lead to lower limb or mesenteric ischemia [4, 5].

Recently, in the CANVAS trial (Canagliflozin Cardiovascular Assessment Study), the use of canagliflozin, compared to placebo, has been associated with doubling of the risk of LLA [6]. Canagliflozin, a sodium/glucose cotransporter 2 (SGLT2) inhibitor, is a glucose-lowering agent with a mild diuretic action consequent to glycosuria-induced osmotic diuresis. A potential mechanism for the increased risk of LLA in CANVAS could be this drug-induced hypovolemia. With this hypothesis, diuretics should also show a similar safety profile. A few previous studies analyzed the association between diuretics use and LLA but were limited regarding type of diuretics or number of participants with diabetes [7, 8].

In this study, we analyzed the association between diuretics and the incidence of lower limb events (LLE), LLA or lower limb revascularization (LLR), in a prospective observational cohort of patients with type 2 diabetes.

METHODS

Study Protocols and Participants

SURDIAGENE study is a French prospective single-center cohort of patients with type 2 diabetes regularly visiting the Diabetes Department at Poitiers University Hospital,

France [9]. Patients aged ≥18 years with a definite diagnosis of T2D for ≥2 years were recruited from 2002 to 2012. Outcome update has been performed prospectively every two years from 2007 till 2015. The main exclusion criteria were non-diabetic kidney disease and follow-up duration <1 month. The Poitiers University Hospital Ethics Committee approved the study design (CPP Ouest III). All participants provided a written informed consent.

Clinical and biological data, including personal medical history and medication use were collected at baseline. Medications use were physician reported and collected at baseline through drug prescription analyses. Diuretics class included thiazide, loop and potassium-sparing diuretics. Mineralocorticoid receptor antagonists were not considered as a diuretic in our analysis. No data were available on the type of diuretics used by each participant and diuretics users were assembled in a single group. Date of the first initiation of treatments was not available in this cohort. A prevalent user design was used here. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula [10].

Clinical outcomes

The primary outcome was the first occurrence of a LLE during follow-up. LLE was defined as a composite of LLA or lower limb revascularization (LLR), whichever came first. LLA was defined as an amputation at or above the metatarsophalangeal joint. LLR was defined as the requirement of peripheral (aorta or lower-limb arteries) revascularization procedure by angioplasty or bypass. None of the reported amputations were traumatic. Each LLE outcome was considered separately as a secondary endpoint. Outcomes were individually determined from patients' hospital records, French death certificate registries, and interviews with their general practitioners. Patients moving out of the hospital area (Poitou–Charentes district) were censored at the time of their departure. An independent adjudication committee reviewed every prospectively collected event [9]. Each endpoint was

reviewed by two independent physicians, and, in case of disagreement, the whole committee discussed the end-point until agreement was found. Dates of event reported by the adjudication committee were the exact date of events.

Statistical analyses

Continuous variables are expressed as mean ± SD, or as median (25th, 75th percentile) for those with skewed distribution. Categorical variables are expressed as the number of participants with corresponding percentage. Comparisons between users and non-users of diuretics were conducted using *t*-test, Wilcoxon, or χ^2 tests.

Participants with at least one missing value of any of the covariates used in the adjusted Cox model were removed from all analyses including this covariate.

Probability of remaining free of LLE and individual components of LLE were plotted as Kaplan-Meier curves according to use of diuretics at baseline and compared using the log-rank test. Cox proportional hazards regression models were fitted to estimate hazard ratios (HR), with associated 95% CI, for endpoints during follow-up for participants taking diuretics compared with participants not on diuretics. We included in our multivariable Cox model all baseline characteristics with $p<0.10$ in the comparison between participants with incident outcomes during follow-up and those with no events : age, diabetes duration, sex, body mass index, systolic blood pressure, hypertension, diabetic retinopathy, previous cardiovascular disease, previous amputation, previous peripheral arterial disease, HDL-cholesterol, albumin to creatinine ratio, eGFR, antiplatelet/anticoagulant, blood pressure lowering agents, statins, biguanides and insulin use (data from ESM Table 1). Due to the high rate of comorbidities in participants prone to LLE, a large proportion of participants may succumb to deaths before LLE occur. Thus, analyses were also adjusted for death considered as a competing risk using the Fine and Gray method [11]. The Schoenfeld residuals method was used to assess the proportional hazards assumption for the association between primary endpoint and diuretics

use (p=0.83).

Since diuretics are preferentially prescribed to patients with heart failure, the latter could lead to allocation bias. However, baseline data for heart failure was not available in our cohort. To overcome this limitation, we did a sensitivity analysis in a subgroup of participants without a congestive heart failure episode occurring during follow up (n=1212), assuming that this group should have a lower prevalence of heart failure at baseline compared with participants experiencing congestive heart failure during follow-up.

We also compared diuretics users to an active comparator class, renin angiotensin aldosterone system (RAAS) blockers (e.g. angiotensin conversion enzyme inhibitors and angiotensin receptor blockers). The choice of this comparator was based on the number of participants taking only one class for comparison and because both classes have opposite action on RAAS which has been shown to be involved in peripheral arterial disease[12].

Finally, to better account for differences in baseline characteristics of participants taking or not diuretics, participants were propensity- score- matched (1:1) based on baseline characteristics using a "nearest neighbor matching" algorithm and a caliper at 0.1 [13]. Baseline characteristics used for matching included: age, sex, diabetes duration, body mass index, smoking, systolic and diastolic blood pressure, history of hypertension, history of cardiovascular disease, history of myocardial infarction, history of LLE, total-cholesterol, HDL-cholesterol, triglycerides, diabetic retinopathy, albuminuria, HbA1c, eGFR, antiplatelet or anticoagulant use, blood pressure lowering drugs use, beta blockers use, RAAS blockers use, statins use and insulin use). We matched 964 participants (71.9% and 61.1% of diuretics users and non-users before matching, respectively). After propensity score matching, all absolute standardized differences were <10%, indicating robust matching [13]. Cox models were fitted for endpoints with diuretics as covariate in the matched cohort.

Statistics were performed with JMP (SAS Institute, Cary, NC) and R software (version

3.4.2). Two-sided p-values less than 0.05 were considered significant.

RESULTS

Baseline characteristics

Among the 1468 participants enrolled in the full cohort, 9 participants were excluded for missing baseline data on diuretics use. Characteristics of participants at baseline are shown in Table 1. Participants taking diuretics at baseline were significantly older, had significantly lower eGFR, higher ACR, used more frequently other blood pressure lowering medications and more often had history of cardiovascular disease compared to those not on diuretics. LLE occurred in 142 participants (9.7%) during follow-up (first event to occur in case of recurrence). During a follow-up of 7.1 years, the incidence rate of LLE was 1.38 per 100 person.years. Taken individually, LLA occurred in 79 (5.4%) and LLR in 98 (6.7%) participants. The incidence rate of LLA and LLR were 0.78 and 0.98 person.years, respectively. Participants who experienced either event, compared with those who did not, were more often males, had longer duration of diabetes, higher rates of complications and previous cardiovascular diseases. They had higher rates of previous amputation and peripheral arterial disease and were more often treated with cardioprotective medications (blood pressure lowering drugs, statins, antiplatelet and anticoagulant agents) (ESM Table 1).

Diuretics use and lower limb events during follow-up

During a median follow-up of 7.1 years, incident rate of LLE was 1.80 per 100 patient.years in diuretics users versus 1.00 in non-users ($p<0.001$). The probabilities of remaining event-free during follow-up according to diuretics use are presented in Figure 1. Cox proportional hazards survival regression analyses showed significant association between the use of diuretics and the incidence of LLE. After adjustment for cofounders, the rate of LLE remained higher in participants on diuretics compared to non-users (Table 2).

Among participants taking or not diuretics at baseline, incident rates for LLA were 1.07 versus 0.50 per 100 patient.years respectively (p<0.001), and for LLR were 1.14 and 0.80 per 100 patient.years respectively (p=0.04). Differences in LLA incidence were not significant depending on the site of amputation (transfemoral, transtibial or transmetatarsal) (p=0.86). Rate of LLA remained significantly increased after adjustment whereas risk of LLR was no longer significant among diuretics users in adjusted model (Table 2).

All-cause death occurred in 542 participants (37.1%) during follow-up, including 78 participants with any LLE during follow-up. In competing risk regression analyses, subhazard ratios for LLE, LLA and LLR were similar to hazard ratio from the Cox model (Table 2), indicating that death was not a competing risk in the association of diuretics with outcomes.

Sensitivity analyses

We calculated hazard ratio in the subgroup of participants without incident episode of congestive heart failure during follow-up (n=1212). Incident rate of LLE and LEA remained significantly higher in participants taking diuretics compared with those not taking diuretics. This increased rate was not observed for LLR (ESM Table 2).

We also compared participants taking diuretics but no RAAS blockers (n=143) to participants taking RAAS blockers but no diuretics (n= 393). Adjusted hazard ratio was 1.84 (95%CI 0.97, 3.47; p= 0.06) for LLE.

After propensity score matching, baseline characteristics between groups were better balanced with no significant difference between diuretics users and non-users (ESM Table 3). In Cox proportional hazards survival regression analyses, we observed a significant increased rate of primary outcomes in participants on diuretics compared with non-diuretics users (Table 3). Similar and significant increase risk of LLA was observed with diuretics use while LLR was not significantly different according to diuretics use (Table 3).

DISCUSSION

In this analysis of 1459 participants with type 2 diabetes, use of diuretics was strongly and independently associated with an increased risk of LLE, and more specifically of LLA. We observed consistent findings after multiple adjustment methods on confounding covariates. Previous epidemiological data have already suggested a similar association in individuals with and without diabetes[7, 8]. The absolute risk of LLE is much higher in long-standing diabetes, but, to our knowledge, this is the first longitudinal study supporting an association between diuretics use and LLE in type 2 diabetes.

The association between antihypertensive medications and LLA in diabetes has been investigated in 450 000 participants in a cross-sectional study on electronic medical records [7]. In line with our results, the authors reported a positive association between thiazides and LLA compared with other antihypertensive drugs. However, the design was cross-sectional and other classes of diuretics (loop and potassium-sparing diuretics) were not included in the analyses. In another cross-sectional study of 1000 participants with known PAD, a 1.5-fold increased risk of critical limb ischemia with use of loop diuretics was reported but no information was available regarding other types of diuretics [8]. Randomized controlled trials testing diuretics would have been the best design to assess causality, but relevant reports did not include precise data on LLE, likely because these events are unfrequently expected to occur in this setting. However, the large Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) showed a trend towards a lower rate of peripheral artery disease events with amlodipine versus chlorthalidone (Hazard Ratio: 0.87 (0.75, 1.01; p=0.06) [14]. The Intervention as a Goal in Hypertension Treatment (INSIGHT) trial reported also a higher incidence of peripheral vascular disorders with co-amilozide versus nifedipine (5.3% vs 3.0%, p<0.0001) [15]. In line with our results, it could be argued that the trend

observed in these trials might be related to a deleterious effect of diuretics rather than a protective action of calcium channel blockers.

While beyond the scope of this observational analysis, we can speculate on the potential underlying mechanism between diuretic exposure and LLE. The most likely mechanism that could explain our results is the extracellular volume depletion induced by diuretics. Indeed, hypovolemia and dehydration could decrease lower extremity perfusion, trigger hemodynamic decompensation and increase blood viscosity, leading to ischemia and amputation. Few cases series of leg or mesenteric ischemia related to extracellular volume deficit induced by diuretics have been reported in the seventies [4, 5]. Similarly, Tzoulaki et al. showed that increased hematocrit, a marker of volemia, was significantly associated with incident peripheral artery disease events[16]. Nonetheless, diuretic induced volume depletion is not similar between the different classes of diuretics. Volume depletion effect is much more important with loop than thiazide diuretics. It would have been useful to compare the effect of each class of diuretic to assess this hypervolemia-driven hypothesis. However, such data were not available in our cohort. Another theoretical mechanism may be the intracellular swelling due to diuretic-induced electrolyte changes which has been shown to contribute to ischemic process [17].

Recently, The CANVAS trial showed that LLA occurred about twice as often in participants treated with canagliflozin compared with placebo [6]. Consistent with this result, in a population-based cohort study of 25 258 propensity matched participants, Udell et al. observed a 2-fold risk of below-knee lower extremity amputation associated with SGLT2 inhibitors initiation [18]. Although mechanisms involved are still unknown, in the light of our results, one potential culprit could be the diuretic-like effect of SGLT2 inhibition. If this side-effect is caused by drug-induced hypovolemia, it should be shared throughout the class. However, whether this risk of amputation is singly with canagliflozin or is a class effect

remains controversial. Recent observational studies and meta-analyses have reported conflicting results [19-27]. Increased risk of LLA was not observed in participants on empagliflozin in the EMPA-REG OUTCOME trial (Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients); however, LLA were identified manually in a post-hoc analysis [28]. Nonetheless, in a recent analysis of nationwide registers from Sweden and Denmark including 34 426 propensity matched participants, use of SGLT2 inhibitors, with only 1% of canagliflozin users, was associated with a 2-fold risk of lower limb amputation compared with GLP1 receptor agonists [27]. Although no data are available regarding a potential different diuretic effect of SGLT2 inhibitors molecules, this could be an explanation of the discrepancy observed for amputation risk in these studies.

This diuresis-driven hypothesis could have been reinforced with a stronger association in participants with PAD at baseline as observed in CANVAS trial and the study of Udell et al [6, 18]. However, the number of such participants in our study was too limited to test this hypothesis (n=73) (ESM Table 1). Larger studies are certainly required for further assessment.

Our study had several limitations. Most of the weakness and limitations were related to the nature of the dataset. Indeed, we analyzed a prospective observational cohort initially created to assess the genetic determinant of diabetic nephropathy and not a drug effect. So, the lack of details on treatments and the potential residual confounding due to the observational nature of our cohort were the main limitations of our study. First, we assessed only baseline exposure to diuretics, leaving some uncertainty with regards to their introduction or interruption, possibly blurring our results. Similarly, since index date for drug initiation was not available, we could not assess time-varying hazards and drug effects associated with treatment duration. Lack of data on date of initiation and interruption of diuretics was a major limitation to our results. Second, because of its observational design,

our study did not allow any direct exploration of a causal relationship between diuretics use and LLE. We are aware that the most relevant method to examine such a deleterious effect of diuretics would be to focus on a new user cohort and compare users of diuretics to users of other non-diuretics antihypertensives to minimize selection bias and immortal time bias. However, despite limited number of participants, we found a similar trend toward a higher risk of LLE with RAAS blockers use. SURDIAGENE cohort was also conducted in a single French diabetes department and may not be representative of all populations with type 2 diabetes. However, since decision of amputation practice is not standardized and likely not similar among different centres, it could lead to bias in such study, so the single centre design (with homogenous practice for amputation) of our cohort can be a point of strength. Our findings can only be generalized for Caucasians people with type 2 diabetes. Moreover, measures to assess volemic status were not available in this cohort to test our volemia hypothesis. Finally, despite multiple adjustments, we cannot avoid the possibility of residual confounding from both potential unmeasured confounders and residual confounding in measured confounders. As baseline characteristics of participants showed a clear higher burden of complications, risk of confounding by indication was high. As sensitivity analysis, we analyzed association between outcomes and diuretics use after propensity score adjustment and propensity score matched sample with well-balanced covariates across both groups. However, despite all the adjustments, we cannot exclude that our findings are more the consequence of an invisible pre-selection of participants instead of a drug effect.

CONCLUSIONS

In conclusion, we consolidate and expand previous findings showing that diuretics are associated with an increased risk of LLA and LLR in participants with type 2 diabetes. Our results could provide a potential explanation for the increased risk of LLA observed with canagliflozin. However, the SURDIAGENE cohort was not designed to assess impact of

drugs on outcomes and our results should be interpreted with caution and further analyses are needed for confirmation.

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Independent adjudication committee of the SURDIAGENE study includes:

Jean Michel Halimi (Chairman Tours, France), Gregory Ducrocq (Paris, France), Ronan Roussel (Paris, France), Pierre Llatty (Poitiers, France), Vincent Rigalleau (Bordeaux, France), Charlotte Hulin (Poitiers, France), David Montaigne (Lille, France) and Philippe Zaoui (Grenoble, France).

Some of the data were presented as an abstract at the EASD meeting in 2018 (Session 7, Abstract 12).

Data availability

The datasets analyzed during the current study are not publicly available due to consideration of intellectual property and continuing analyses by the study investigators but are available from the corresponding author upon reasonable request.

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Duality of interest

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Contribution statement

LP had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. LP, RR, KM, and SH made substantial contributions to conception and design of the study. All authors made substantial contributions to acquisition, analysis, or interpretation of data and gave final approval of the version to be published. LP and RR drafted the manuscript and all the authors made critical revision of the manuscript for important intellectual content.

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	No diuretic	Diuretic	p	missing data n (%)
n	789	670		
Age, years	62.9 ± 11.1	67.1 ± 9.5	<0.001	0
Diabetes duration, years	13.1 ± 9.5	16.2 ± 10.2	<0.001	2 (0.1)
Sex (Male), n (%)	468 (59.3)	374 (55.8)	0.20	0
BMI, kg/m^2	30.7 ± 6.1	32.0 ± 6.4	<0.001	0
Current smokers, n (%)	99 (12.5)	54 (8.1)	0.02	18 (1.0)
Heart rate, bpm	71.6 ± 13.7	70.4 ± 14.0	0.12	8 (0.5)
Systolic blood pressure, mmHg	131.9 ± 17.1	133.2 ± 18.5	0.16	7 (0.5)
Diastolic blood pressure, mmHg	72.8 ± 10.9	71.9 ± 11.4	0.12	7 (0.5)
Hypertension, n (%)	540 (68.4)	668 (99.7)	<0.001	0
Renal replacement therapy, n (%)	12 (1.5)	9 (1.3)	0.95	0
Diabetic retinopathy, n (%)	328 (41.6)	311 (46.4)	0.07	6 (0.4)
Previous cardiovascular disease, n (%)	185 (23.4)	218 (32.5)	<0.001	0
Myocardial infarction	102 (12.9)	122 (18.2)	0.01	0
Coronary revascularization	98 (12.4)	121 (18.1)	0.003	0
Stroke	43 (5.4)	40 (6.0)	0.75	0
Carotid revascularization	14 (1.8)	21 (3.1)	0.13	0
Peripheral arterial disease	33 (4.2)	40 (6.0)	0.15	0
History of amputation, n (%)	31 (3.9)	42 (6.3)	0.06	0
Amputation level, n (%)				
Toe	16 (2.0)	23 (3.4)		0
Transmetatarsal	8 (1.0)	7 (1.0)		0
Transtibial	5 (0.6)	3 (0.4)		0
Transfemoral	2 (0.3)	9 (1.3)		0
HbA1c, % and mmol/mol	7.8 ± 1.6 61.9 ± 17.5	7.7 ± 1.5 61.1 ± 16.1	0.40	1 (0.06)
Total cholesterol, mmol/l	4.9 ± 1.1	4.7 ± 1.2	0.002	0
LDL- cholesterol, mmol/l	2.8 ± 0.9	2.6 ± 1.0	<0.001	59 (4.0)
HDL- cholesterol, mmol/l	1.2 ± 0.4	1.2 ± 0.4	0.35	8 (0.5)
Triglycerides. mmol/l[a]	1.6 [1.1. 2.2]	1.6 [1.2. 2.4]	0.07	5 (0.3)
ACR, mg/mmol[a]	2.4 [0.9. 9.9]	4.1 [1.2. 19.2]	<0.001	15 (1.0)
eGFR, mL/min/1.73m^2	77.6 ± 23.9	66.5 ± 25.2	<0.001	0
Antiplatelet or anticoagulant agent, n (%)	309 (39.2)	308 (46.0)	0.01	0
Blood pressure lowering treatments, n (%)	539 (68.3)	670 (100.0)	<0.001	0
RAAS blockers, n (%)	393 (49.8)	527 (78.7)	<0.001	0
Calcium channel blockers, n (%)	201 (25.5)	262 (39.1)	<0.001	0
Beta-blockers, n (%)	203 (25.7)	296 (44.2)	<0.001	0
Statins, n (%)	312 (39.5)	353 (52.7)	<0.001	0
Biguanides, n (%)	396 (50.2)	286 (42.7)	0.01	0
Sulfonylureas, n (%)	338 (42.8)	244 (36.4)	0.02	0
Thiazolidinediones, n (%)	11 (1.4)	6 (0.9)	0.52	0
Alpha glucosidase inhibitor, n (%)	52 (6.6)	28 (4.2)	0.06	0
Insulin, n (%)	455 (57.7)	425 (63.4)	0.03	0

1

Table 1: Characteristics of participants at baseline according to diuretics use. Data expressed as mean ± SD except ([a]) expressed as median (25% - 75% percentile). Statistics for quantitative characteristics are t-test with log-transformed data, except ([a]) Wilcoxon test. Hypertension: systolic blood pressure (SBP) >140 mmHg and/or diastolic blood pressure (DBP) >90 mmHg or presence of antihypertensive medication and history of hypertension. ACR: urine albumin-to-creatinine ratio. BMI: Body Mass Index. eGFR: estimated glomerular filtration rate. RAAS: renin angiotensin aldosterone system.

	LLE		LLA		LLR	
	Hazard Ratio (95% CI)	***P***	**Hazard Ratio (95% CI)**	***P***	**Hazard Ratio (95% CI)**	***P***
Unadjusted	2.08 (1.49, 2.93)	<0.001	2.53 (1.61, 4.10)	<0.001	1.71 (1.15, 2.56)	0.01
Adjusted [a]	1.49 (1.01, 2.19)	0.04	2.01 (1.14, 3.54)	0.02	1.05 (0.67, 1.64)	0.84
	Subhazard Ratio (95% CI)	***P***	**Subhazard Ratio (95% CI)**	***P***	**Subhazard Ratio (95% CI)**	***P***
Death as competing risk [b]	1.89 (1.35, 2.64)	<0.001	2.28 (1.44, 3.62)	<0.001	1.52 (1.02, 2.27)	0.04

Table 2: Hazard ratio of lower limb events (LLE), lower limb amputation (LLA) and lower limb revascularization (LLR) during follow-up associated with the use of diuretics at baseline. [a] Adjusted for age, diabetes duration, sex, BMI, SBP, hypertension, diabetic retinopathy, previous cardiovascular disease, previous amputation, previous peripheral arterial disease, HDL-cholesterol, albumin to creatinin ratio, eGFR, antiplatelet/anticoagulant, blood pressure lowering agents, statins, biguanides and insulin use. [b] Fine and Gray model (competing risk = all-cause death). Number of individuals included in the Cox analyses: 1459 in the unadjusted analysis, 1418 in the adjusted model and 1459 in the Fine and Gray model.

LLE		LLA		LLR	
Hazard Ratio (95% CI)	**p**	**Hazard Ratio (95% CI)**	**p**	**Hazard Ratio (95% CI)**	**p**
1.53 (1.05, 2.24)	0.03	1.98 (1.15, 3.40)	0.01	1.11 (0.72, 1.71)	0.65

Table 3: Hazard ratio of lower limb events (LLE), lower limb amputation (LLA) and lower limb revascularization (LLR) during follow-up associated with the use of diuretics at baseline in the propensity score matched cohorts.

FIGURE LEGEND

Figure 1: Kaplan-Meier curves for cumulative outcomes free survival during follow-up with and without diuretics use. A: lower limb events (LLA or LLR). B: lower limb amputation (LLA). C: lower limb revascularization (LLR). Log-rank test: $p<0.001$.