Sodium Glucose Co-transporter-2 Inhibitors and the Risk of Below-knee Amputation: a Multicenter Observational Study

Running title: Sodium glucose co-transporter-2 inhibitors and amputation

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ABSTRACT

Objective: Reports of amputations associated with sodium glucose co-transporter (SGLT) 2 inhibitors have been inconsistent. We aimed to compare the risk of below-knee amputation with SGLT2 inhibitors versus dipeptidyl peptidase (DPP)-4 inhibitors among patients with type 2 diabetes.

Research Design and Methods: This is a multicenter observational study using administrative healthcare databases from 7 Canadian provinces and the United Kingdom. Incident SGLT2 inhibitor users were matched to DPP-4 inhibitor users using a prevalent new user design and time-conditional propensity scores. Cox proportional hazards models were used to estimate site-specific adjusted hazard ratios (HR) and corresponding 95% confidence intervals (CI) of incident below-knee amputation for SGLT2 inhibitor versus DPP-4 inhibitor users. Random effects meta-analyses were used to pool the site-specific results.

Results: The study cohort included 207,817 incident SGLT2 inhibitor users matched to 207,817 DPP-4 inhibitor users. During a mean exposed follow up time of 11 months, the amputation rate among SGLT2 inhibitor users was 1.3 per 1,000 person-years and 1.5 per 1,000 person-years among DPP-4 inhibitor users. The adjusted HR of below-knee amputations associated with SGLT2 inhibitor use compared to DPP-4 inhibitor use was 0.88 (95% CI: 0.71-1.09). Similar results were obtained in stratified analyses by specific SGLT2 inhibitor molecule.

Conclusions: In this large multicenter observational study, there was no association between SGLT2 inhibitor use and incident below-knee amputations among patients with type 2 diabetes, compared to DPP-4 inhibitor use. While these findings provide some reassurance, studies with longer duration of follow-up are needed to assess potential long-term effects.

INTRODUCTION

Sodium glucose co-transporter (SGLT) 2 inhibitors are the newest anti-diabetic agents for type 2 diabetes management (1). They inhibit the sodium-glucose co-transporters on renal proximal tubules, leading to glucosuria. This effect not only lowers glycemia but also induces weight loss and blood pressure reduction (2). Indeed, randomized placebo-controlled trials have shown that SGLT2 inhibitors also decrease the risk of cardiovascular outcomes (3; 4) and heart failure (3-5). Current guidelines from the American Diabetes Association (ADA) recommend the use of SGLT2 inhibitors as second or third line in addition to metformin in the management of type 2 diabetes (1). Due to the cardiovascular and renal benefits conferred by these agents, SGLT2 inhibitors are recommended as one of the preferred second line agents for patients who have high risk factors or known cardiovascular disease, heart failure and chronic renal disease (i.e. estimated glomerular filtration rate [eGFR] of 30-60 mL/min/1.73m^2 or urine albumin-to-creatinine ratio of 30mg/g). Given the cardiovascular benefits of SGLT2 inhibitors and the emphasis on these benefits in recent treatment guidelines, the use of SGLT2 inhibitors has increased substantially among patients with type 2 diabetes (6).

Despite the cardiovascular benefits associated with SGLT2 inhibitor treatment, there are several safety concerns associated with their use, including a reported increased risk of below-knee amputations (3; 7). Concerns regarding this adverse event stem from the CANVAS Program trial, in which participants randomized to the SGLT2 inhibitor canagliflozin had a two-fold increased risk compared to participants randomized to placebo (hazard ratio [HR]: 1.97; 95% confidence interval [CI]: 1.41-2.75)(3). As a result of this finding, the United States Food and Drug Administration (FDA) issued a black-box warning of amputation risk for canagliflozin (8). However, there was no increased risk of amputation associated with canagliflozin use among patients with type 2 diabetes and chronic renal disease compared to patients treated with placebo

in the CREDENCE trial, which assessed canagliflozin use and renal outcomes (HR:1.11; 95% CI: 0.79-1.56) (9). Nevertheless, two pharmacovigilance analyses found an increased reporting of amputation among individuals treated with SGLT2 inhibitors compared to other anti-diabetic agents (10; 11). Several observational studies have been conducted to assess the association between SGLT2 inhibitor use and the risk of amputation, with the majority of studies using the same employment insurance database in the United States; however, their findings have been inconsistent (7; 12-18).

In light of the findings from the CANVAS Program trial and the inconsistent results of previous observational studies, further studies are needed to address whether SGLT2 inhibitor use is associated with an increased risk of amputation. Using data from Canada and the United Kingdom, the aim of this study was to determine whether SGLT2 inhibitor use compared with DPP-4 inhibitor use, is associated with an increased risk of below-knee amputation among patients with type 2 diabetes in a real-world setting.

METHODS

Source population

We used administrative healthcare databases from the Canadian provinces of Alberta, British Columbia, Manitoba, Nova Scotia, Ontario, Quebec, and Saskatchewan, and the United Kingdom (UK) Clinical Practice Research Datalink (CPRD). The Canadian databases contain population-level data on physician claims, hospitalization records and prescription drug claims. Prescription drug data are only available for individuals aged ≥18 years in Alberta, aged ≥65 years in Ontario, and those ≥65 years, receiving social assistance and without access to private drug insurance in Quebec.

The CPRD is a large primary care database containing medical information documented by primary care physicians on approximately 13 million patients enrolled in over 680 general practices in the UK (19; 20). This database documents demographic characteristics, diagnoses, laboratory test results, procedures, prescriptions, medical history, administrative information and clinical data, including smoking, body mass index (BMI) and alcohol use. The CPRD is regularly audited and the data has been shown to be valid and of high quality (19; 21). CPRD data were linked to the Hospital Episodes Statistics (HES) database, which contains full hospitalization data from 1997 to the present. HES linkage for this study was from April 1, 1997 to December 31, 2017.

The study protocol was registered in clinicaltrials.gov (https://clinicaltrials.gov/ct2/show/NCT04017221). Ethics approval was obtained at each participating site. The study protocol received ethical and scientific approval from the Independent Advisory Scientific Committee of the CPRD (protocol number: 19_007A2).

Individuals aged 18 years or older with type 2 diabetes were identified by prescriptions for an anti-diabetic medication (alpha-glucosidase inhibitors, DPP-4 inhibitors, glucagon-like peptide [GLP]-1 receptor agonists, insulin, meglitinides, metformin, SGLT2 inhibitors, sulfonylureas, thiazolidinediones, or combinations of these drugs) between January 1, 2006 and June 30, 2018 or the most recent date of data availability at each site. Potentially eligible subjects were identified as of 2006 to cover the period of general availability of DPP-4 inhibitors and SGLT2 inhibitors. In Nova Scotia, due to limitations in prescription drug data availability, individuals who received anti-diabetic medications between November 1, 2017 and June 30, 2018 were eligible to be included in the study cohort.

From this source population, we constructed the study cohort of users of SGLT2 inhibitors and users of DPP-4 inhibitors with a prescription after the date of introduction of SGLT2 inhibitors at each study site (Appendix Table 1). Identifiable information was not accessible and we complied with all privacy requirements of the data custodians at each site. A prevalent new user design (22) was used to match each user of an SGLT2 inhibitor to a user of a DPP-4 inhibitor. Study cohort entry among SGLT2 inhibitor users was defined by the date of the first SGLT2 dispensing (or prescription in CPRD). Study cohort entry for DPP-4 inhibitor users was the date of the matched (see below) DPP-4 inhibitor prescription during the period defined by the first prescription of SGLT2 inhibitors and June 30, 2018. Individuals with a prior history of amputation at any time prior to or on study cohort entry were excluded.

Matching

We created exposure sets that were defined by user type (incident versus prevalent), level of anti-diabetic treatment, prior use of GLP-1 agonists, and calendar time (DPP-4 inhibitor prescription within 120 days of the SGLT2 inhibitor initiation). Incident users were defined as using SGLT2 inhibitor or DPP-4 inhibitor for the first time (i.e. new users). Incident SGLT2 inhibitor users were matched to incident DPP-4 inhibitor users. Patients treated with DPP-4 inhibitors who switched to or added a SGLT2 inhibitor to their treatment regimen (prevalent new users) were matched to patients treated with DPP-4 inhibitors for the same duration but who remained on DPP-4 inhibitor treatment (Appendix Figure 1). We also matched on the level of anti-diabetic treatment, a three-level categorical variable created as a proxy for severity of diabetes. Three levels of anti-diabetic treatment were created to mirror the severity of type 2 diabetes based on the type and number of different anti-diabetic agents in the prior 365 days. The first level was defined as patients treated with only one anti-diabetic agent or treated with lifestyle modifications

(i.e., they did not receive an anti-diabetic agent during the prior 365 days). The second level included patients that required at least two non-insulin anti-diabetic agents. Finally, the third level included patients who received insulin treatment (either alone or in combination with other anti-diabetic agents). Time conditional propensity scores (TCPS) were then constructed separately for incident and prevalent new users, using conditional logistic regression stratified by exposure set to estimate the propensity of receiving a SGLT2 inhibitor versus a DPP-4 inhibitor using covariates shown below (please see covariate section). Scores were computed for each individual in each exposure set; hence, an individual may have different scores for exposure sets they enter, depending on the time of entry (i.e., time conditional). Additional covariates were included in the TCPS in the CPRD cohort (please see covariate section). SGLT2 inhibitor users were matched 1:1 without replacement to users of DPP-4 inhibitors from their exposure set with the closest TCPS and in chronological order. However, in 5 sites, there was a loss of >10% of exposure sets after trimming the areas of non-overlap of the TCPS distribution and matching. In these sites, matching with replacement was performed using a caliper width of ±0.2 standard deviations of log TCPS.

Covariates

The following covariates, defined *a priori*, were used to construct the TCPS in all study cohorts included age, sex, calendar year at cohort entry, and diabetes duration (<1 year, 1-4.9 years, 5-10 years, and >10 years). We included comorbidities identified during the 3 years prior to study cohort entry such as alcohol-related disorders, cancer, cerebrovascular disease, cirrhosis, coronary artery disease, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, dialysis, hypertension, ischemic stroke, myocardial infarction, other kidney diseases, and peripheral arterial disease. We also included medication use in the year prior to study cohort such as acetylsalicylic acid, aldosterone antagonists, alpha-glucosidase inhibitors, angiotensin II receptor blockers,

angiotensin-converting enzyme inhibitors, beta-blockers, calcium channel blockers, digitalis-like agents, direct renin inhibitors, GLP-1 receptor agonists, insulin, loop diuretics, meglitinides, metformin, non-acetylsalicylic acid antiplatelet drugs, nonsteroidal anti-inflammatory drugs, oral anticoagulants, other diuretics, other lipid lowering therapy, statins, sulfonylureas, thiazide diuretics, and thiazolidinediones. Finally, we included covariates that are indicators of health care use in the year prior to study cohort entry including the number of inpatient hospitalizations (0, 1-2, and ≥3) and number of physician visits (0-2, 3-5, and ≥6).

In the CPRD study cohort, additional *a priori* defined covariates were included in the TCPS models. These covariates included body mass index, smoking status (never, ever, and unknown), race, blood pressure, estimated glomerular filtration rate, and glycated hemoglobin A1c (HbA1c).

Exposure assessment

Patients were classified as being current users of SGLT2 inhibitors (canagliflozin, dapagliflozin, empagliflozin) alone or in combination with other anti-diabetic agents or current users of DPP-4 inhibitors (alogliptin, linagliptin, saxagliptin, sitagliptin, vildagliptin) alone or in combination with non-SGLT2 inhibitor anti-diabetic agents. Exposure was defined using an as-treated approach whereby exposure was time-fixed and defined by the cohort entry drug. Patients were followed until they discontinued treatment defined as a gap of 30 or more days after the end of a prescription (grace period) or the initiation of an SGLT2 inhibitor for users of DPP-4 inhibitors, or censored due to death, end of healthcare coverage, or end of study period, whichever occurred first. DPP-4 inhibitor use was the comparator as this class of anti-diabetic agent is also a second or third line treatment and has not been shown to be associated with increased amputation risk (23).

Study outcomes

The primary study outcome was incident below-knee amputation defined as having transtibial amputations, or amputations involving the ankle and foot using procedure codes documented during hospitalization or physician claims data (24). Below-knee amputation was assessed given that 71% of amputations that occurred in the CANVAS Program trial were at the toe and metatarsal level (3; 25). Furthermore, given that this study excluded individuals with prior history of amputation, it would be clinically unlikely for individuals to develop incident above knee amputation associated with SGLT2 inhibitor use. The diagnostic and procedure codes used to define this outcome are reported in Appendix Table 2.

Statistical analyses

Patient characteristics were summarized in each study cohort using frequencies and percentages for categorical variables and means (standard deviation) for continuous variables. Multivariable Cox proportional hazards models were used to estimate site-specific adjusted HR and corresponding 95% CI for the risk of incident below-knee amputation among SGLT2 inhibitor users versus DPP-4 inhibitor users. Cox models were adjusted for age, sex, diabetes duration and deciles of TCPS. In secondary analyses, we stratified by age to determine if the risk of amputation associated with SGLT2 inhibitor use was higher among the elderly population (≥70 and <70 years), sex, prior insulin use (defined as insulin use in the previous year), and SGLT2 inhibitor molecule. Finally, we completed five sensitivity analyses. First, we repeated the primary analysis using grace periods of 0, 60, and 365 days to define continuous use of study drug. Second, we stratified the primary analysis by incident and prevalent new user status among SGLT2 inhibitor users. Third, we defined exposure using an intention-to-treat approach in which exposure was defined at cohort entry and patients were followed until occurrence of the outcome or censored due to death, end of healthcare coverage, end of study period, entry into the SGLT2 inhibitor cohort for DPP-4 inhibitor

users, or a maximum of 1 year of follow-up, whichever occurred first. Fourth, given that SGLT2 inhibitor use is not indicated for patients on dialysis, we repeated the primary analysis after excluding individuals with a prior history of dialysis using the study cohort from Ontario as this cohort contained the highest number of individuals with a prior history of dialysis. Kaplan-Meier curves were visually evaluated at each site, to assess potential departures from the assumption of proportional hazards. This assessment revealed no indication that the assumption was violated at any of the sites. Finally, we repeated the primary analysis using a fixed-effects model.

Meta-analysis

We pooled the adjusted HRs from each site using DerSimonian and Laird random-effects meta-analysis with inverse variance weighting (26). Inclusion in the meta-analysis was restricted to sites with at least 5 events in each exposure group (Appendix Table 3). Between-site heterogeneity was assessed using the I^2 statistic. Analyses were conducted using RevMan software version 5.3.

RESULTS

The study cohort included 207,817 SGLT2 inhibitor users matched to 207,817 DPP-4 inhibitor users (Figure 1). Among the users of SGLT2 inhibitors, 102,263 were classified as incident new users and 105,554 as prevalent new users. Baseline characteristics were well balanced after TCPS matching (Table 1, Appendix Table 4). In the CPRD, the number of patients with renal insufficiency, defined as having an eGFR <60 mL/min/1.73m^2 was higher among DPP-4 inhibitor users compared to SGLT2 inhibitor users (Appendix Table 4).

The mean exposed follow-up time for the matched cohort was 11 months (standard deviation: 9 months), generating 369,458 person-years of observation. The rate of incident below-knee amputation was similar among SGLT2 inhibitor users (1.3 per 1,000 person-years) versus DPP-4 inhibitor users (1.5 per 1,000 person-years) in the matched cohort. There was no significant

increased risk of incident below-knee amputation associated with SGLT2 inhibitor use compared to DPP-4 inhibitor use (HR: 0.88; 95% CI: 0.71-1.09; I^2: 18%) (Table 2, Figure 2).

In secondary analyses, the risk of incident below-knee amputation associated with SGLT2 inhibitor use versus DPP-4 inhibitor use did not differ according to age (≥70 versus <70 years), sex, history of prior insulin use or SGLT2 inhibitor molecule (Table 3). The results remained consistent across most sensitivity analyses, including among patients with no prior history of dialysis (Appendix Table 5). However, there was a trend towards an increased risk of incident below-knee amputation among prevalent new users of SGLT2 inhibitors versus DPP-4 inhibitor users (HR: 1.29; 95% CI: 0.97-1.70) (Table 3).

DISCUSSION

In this large, multicenter observational study, using administrative data from seven Canadian provinces and the UK CPRD, we found no increased risk of incident below-knee amputation associated with SGLT2 inhibitor versus DPP-4 inhibitor use among patients with type 2 diabetes (HR: 0.88; 95% CI: 0.71-1.09). Results were consistent across subgroups defined by age, sex, and prior insulin use. Similarly, there was no increased risk of below-knee amputation associated with individual SGLT2 inhibitor use, including canagliflozin compared to DPP-4 inhibitor use.

Concerns regarding an increased risk of amputation associated with the use of SGLT2 inhibitors arose when the CANVAS Program trial found a nearly two-fold increased risk associated with canagliflozin use versus placebo (HR: 1.97; 95% CI: 1.41-2.75) (3). In light of this finding, further analyses were conducted in the EMPA-REG trial to assess amputation risk (27). These analyses revealed no increased risk of amputation with empagliflozin (HR: 1.00; 95% CI: 0.70-1.44) (27). However, as acknowledged by the authors, the ascertainment of amputations may

have been inaccurate, and the results of such post-hoc analyses must be interpreted with caution. In the DECLARE trial, there was no increased risk of amputation associated with randomization to dapagliflozin versus placebo (HR: 1.09; 95% CI: 0.84-1.40) (5). Recently, the CREDENCE trial, which enrolled patients with type 2 diabetes and chronic renal disease to study renal outcomes associated with canagliflozin use, did not find an increased risk of amputation among patients treated with canagliflozin (HR: 1.11; 95% CI: 0.79-1.56) (9).

The safety signal regarding an increased risk of amputation identified in the CANVAS Program trial was supported by pharmacovigilance analyses conducted using the FDA Adverse Event Reporting System (FAERS), which found an increased reporting of amputation specifically among canagliflozin users (proportional reporting ratio [PRR]: 5.33; 95% CI: 4.04-7.04) compared to users of non-SGLT2 inhibitor antidiabetic agents (10). Subsequently, another pharmacovigilance analysis conducted using the World Health Organization (WHO) global database of individual case safety reports (Vigibase) found that the PRR was increased for all available SGLT2 inhibitors compared to other anti-diabetic medications (canagliflozin, PRR: 7.09; 95% CI: 5.25-9.57; empagliflozin, PRR: 4.96; 95% CI: 2.89-8.50 and for dapagliflozin, PRR for toe-amputations: 2.62; 95% CI: 1.33-5.14) (11). Nevertheless, there are well recognized limitations to using adverse event reporting data, which include difficulties with adverse event recognition, underreporting of adverse events, absence of a denominator, biases that affect event reporting and variations in report quality (28).

To date, there have been eight observational studies to our knowledge that have assessed the risk of amputation associated with SGLT2 inhibitor use; these studies have produced heterogeneous results (7; 12-17). Six of these studies utilized the Marketscan Commercial Claims and Encounters Database (CCAE) with five studies showing no association between SGLT2

inhibitor use and amputation (12-15) and one study showing an increased risk of amputation with SGLT2 inhibitor use compared to DPP-4 inhibitor use (HR: 1.69; 95% CI: 1.20-2.38)(7). Interestingly, in this study, SGLT2 inhibitor use was not associated with an increased risk of lower extremity amputation when compared to sulfonylurea use (7). Udell et al.(16) performed an observational study using the United States Department of Defense Health System and found that patients initiating an SGLT2 inhibitor had a nearly 2-fold increased risk of lower extremity amputation compared to patients treated with non-SGLT2 inhibitor anti-diabetic agents (HR: 1.99; 95% CI: 1.12-3.51). However, the majority of amputations in the SGLT2 inhibitor group were among patients treated with canagliflozin. This finding was also observed in the study performed by Ueda et al.(17), using the Swedish/Danish National Register, which found that SGLT2 inhibitor users had an increased risk of incident amputation compared to GLP-1 receptor agonist users (HR: 2.48; 95% CI: 1.14-5.40). The heterogeneity of the findings of these previous studies may be due to a number of factors including differences in the populations assessed, methodologies used, comparator drug used, differences in the extent of amputation (i.e. some studies included above knee amputations (14; 18)), duration of follow-up time, and the inclusion of patients with prior history of amputation (7; 13; 17; 18). Some of these inconsistencies were observed within the same study when the researchers varied the exclusion criteria for their study population (i.e., excluding patients with prior history of amputation, insulin use, renal insufficiency and baseline cardiovascular disease) and when different comparators were used in the analyses (7).

Our study has several strengths including the use of DPP-4 inhibitors as comparators as they are prescribed at a similar stage of type 2 diabetes as SGLT2 inhibitors (i.e., as second or third line). The use of the prevalent new user design allowed inclusion of patients who switched to or added a SGLT2 inhibitor, which is reflective of clinical practice and allows our findings to be more

generalizable to the realities of clinical practice. Indeed, we noted approximately 50% of SGLT2 inhibitor users had previously used DPP-4 inhibitors. Finally, with data from eight databases across two countries, our study is the largest observational study conducted examining this safety issue to date, increasing the precision and generalizability of our results.

Our study also has potential limitations. First, residual confounding is possible given that this is an observational study. However, we used various approaches to minimize confounding by using an active comparator and extensive matching. Second, there is potential confounding by contra-indication as physicians may have been less likely to prescribe SGLT2 inhibitors to patients who are at higher risk for amputation. Furthermore, there is an imbalance in the proportion of SGLT2 inhibitor versus DPP-4 inhibitor users with renal insufficiency (eGFR<60 mL/min/1.73m^2) noted in the CPRD cohort, as use of SGLT2 inhibitors is not recommended for patients with significant renal insufficiency (i.e. eGFR<45 mL/min/1.73m^2) (1; 29). Although the eGFR findings from the CPRD comprised of a small percentage of the total cohort (3.3% of the weight of the meta-analysis) there may be residual confounding as patients with renal insufficiency have a higher risk of amputation compared to patients with normal renal function (30). As such, DPP-4 inhibitor users may have an underlying higher risk of amputation compared to SGLT2 inhibitor users and this could mask the higher risk of amputation associated with SGLT2 inhibitor use. However, in our sensitivity analysis, our results remained consistent among patients with no prior history of dialysis (i.e. during the 3 years prior to study cohort entry). Third, the databases used in this study capture dispensing of medications (i.e. Canadian databases) or prescriptions (i.e. CPRD) without any guarantee that these medications were taken by the patient. Fourth, we did not study the specific site of below-knee amputation since we anticipated insufficient events per site. Fifth, we included users of SGLT2 inhibitors who had (prevalent new users) and had not (incident new

users) used DPP4 inhibitors previously, which may consist of individuals that differ in the severity of diabetes and risk for complications associated with diabetes. In stratified analyses, the risk of below-knee amputation associated with SGLT2 inhibitor use compared to DPP-4 inhibitor use was higher among prevalent new users of SGLT2 inhibitors but this did not reach statistical significance. Finally, the duration of follow up was modest and future studies with longer duration of follow-up are needed to determine if long-term use of SGLT2 inhibitors is associated with below-knee amputation risk as the increased risk of amputation associated with canagliflozin treatment in the CANVAS Program trial was observed near the end of the study period (mean follow-up of approximately 3.6 years) (3). Furthermore, our study focused on patients with no prior history of amputation whereas the CANVAS Program trial and the CREDENCE trial involved patients with a prior history of amputation. Thus, the patients in these trials have an underlying higher risk of amputation compared to the population cohort of our study (rate of amputation in our study was 1.3 per 1000 person-years versus 6.3 per 1000 person-years in the CANVAS Program trial and 12.3 per 1000 person-years in the CREDENCE trial) (3; 9).

In conclusion, in this multicenter observational study, we found no evidence of an association between SGLT2 inhibitor use and incident below-knee amputation compared to DPP-4 inhibitor use among patients with type 2 diabetes. Similarly, there was no increased risk of below-knee amputation associated with specific SGLT2 inhibitor molecule use compared to DPP-4 inhibitor use. Future studies will be needed to further address whether SGLT2 inhibitor use increases the risk of incident below-knee amputation over the longer term.

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DISCLOSURES

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Table 1: Baseline characteristics of users of SGLT2 inhibitors and their matched DPP-4 users*

	SGLT2 inhibitors (n = 207,817)	DPP-4 inhibitors (n = 207,817)
Age (years)	63.8 ± 9.5	64.0 ± 9.6
18-35	3,479 (1.7)	3,612 (1.7)
36-45	12,288 (5.9)	11,915 (5.7)
46-55	30,845 (14.8)	30,105 (14.5)
56-65	47,711 (23.0)	48,079 (23.1)
66-75	89,410 (43.0)	87,993 (42.3)
76-85	22,074 (10.6)	23,920 (11.5)
>85	2,010 (1.0)	2,193 (1.1)
Females	86,360 (41.6)	87,030 (41.9)
Calendar year at cohort entry		
2013	320 (0.2)	343 (0.2)
2014	6,954 (3.3)	7,322 (3.5)
2015	51,464 (24.8)	50,921 (24.5)
2016	66,242 (31.9)	66,422 (32.0)
2017	61,291 (29.5)	61,013 (29.4)
2018	21,546 (10.4)	21,796 (10.5)
New user status		
Incident users	102,263 (49.2)	102,263 (49.2)
Prevalent users	105,554 (50.8)	105,554 (50.8)
SGLT2 inhibitor molecule		
Canagliflozin	87,922 (42.3)	–
Dapagliflozin	63,792 (30.7)	–
Empagliflozin	56,103 (27.0)	–
Diabetes duration (years)	12.6 ± 6.6	12.5 ± 6.6
<1 year	7,166 (3.4)	7,341 (3.5)
1-4.9 years	25,204 (12.1)	25,766 (12.4)
5-10 years	52,543 (25.3)	52,758 (25.4)
>10 years	122,904 (59.1)	121,952 (58.7)
Comorbidities†		
Alcohol-related disorders	3,626 (1.7)	3,658 (1.8)
Cancer	21,692 (10.4)	21,937 (10.6)
Cerebrovascular disease	9,892 (4.8)	10,156 (4.9)
Cirrhosis	3,621 (1.7)	3,606 (1.7)
Coronary artery disease	44,710 (21.5)	43,939 (21.1)
Diabetic nephropathy	7,476 (3.6)	7,478 (3.6)
Diabetic neuropathy	3,807 (1.8)	3,844 (1.8)
Diabetic retinopathy	5,266 (2.5)	5,296 (2.5)
Dialysis	277 (0.1)	315 (0.2)
Hypertension	111,130 (53.5)	111,332 (53.6)
Ischemic stroke	2,448 (1.2)	2,535 (1.2)
Myocardial infarction	5,326 (2.6)	5,113 (2.5)

	SGLT2 inhibitors (n = 207,817)	DPP-4 inhibitors (n = 207,817)
Other kidney diseases	10,222 (4.9)	10,850 (5.2)
Peripheral arterial disease	4,472 (2.2)	4,471 (2.2)
Use of medications[†]		
Acetylsalicylic acid	36,875 (17.7)	36,792 (17.7)
Aldosterone antagonists	6,146 (3.0)	6,182 (3.0)
Alpha-glucosidase inhibitors	3,057 (1.5)	2,949 (1.4)
Angiotensin II receptor blockers	66,747 (32.1)	66,301 (31.9)
Angiotensin-converting enzyme inhibitors	94,489 (45.5)	94,092 (45.3)
Beta-blockers	58,854 (28.3)	58,371 (28.1)
Calcium channel blockers	63,281 (30.5)	63,671 (30.6)
Digitalis-like agents	2,586 (1.2)	2,624 (1.3)
Direct renin inhibitors	104 (0.1)	92 (0.0)
GLP-1 receptor agonists	8,464 (4.1)	8,464 (4.1)
Insulin	57,143 (27.5)	57,143 (27.5)
Loop diuretics	21,314 (10.3)	21,559 (10.4)
Meglitinides	4,680 (2.3)	4,707 (2.3)
Metformin	180,662 (86.9)	180,828 (87.0)
Non-acetylsalicylic acid antiplatelet drugs	14,034 (6.8)	13,655 (6.6)
Nonsteroidal anti-inflammatory drugs	40,470 (19.5)	40,263 (19.4)
Oral anticoagulants	13,393 (6.4)	13,359 (6.4)
Other diuretics	18,497 (8.9)	18,406 (8.9)
Other lipid lowering therapy	23,524 (11.3)	22,937 (11.0)
Statins	159,742 (76.9)	159,061 (76.5)
Sulfonylureas	108,451 (52.2)	108,327 (52.1)
Thiazide diuretics	45,019 (21.7)	44,788 (21.6)
Thiazolidinediones	5,175 (2.5)	4,863 (2.3)
Number of different classes of non-antidiabetic drugs[‡]		
0-1	8,465 (4.1)	8,666 (4.2)
2-5	65,919 (31.7)	66,729 (32.1)
≥6	133,433 (64.2)	132,422 (63.7)
Health care use[†]		
Inpatient hospitalizations		
0	176,833 (85.1)	176,723 (85.0)
1-2	28,687 (13.8)	28,697 (13.8)
≥3	2,296 (1.1)	2,398 (1.2)
Number of physician visits		
0-2	14,963 (7.2)	15,117 (7.3)
3-5	31,883 (15.3)	32,206 (15.5)
≥6	160,971 (77.5)	160,494 (77.2)

Abbreviations: DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; SD, standard deviation; SGLT2, sodium-glucose cotransporter 2

[*]Data are presented as n (%) or mean ± SD. SGLT2 inhibitors patients were matched to DPP-4 inhibitors patients from their exposure-set (defined on level of anti-diabetic therapy, prior use of GLP-1 receptor agonists, time on DPP-4 inhibitors for prevalent new users and calendar time) on time-conditional propensity score. Due to privacy restrictions, value <6 were replaced by 3 before pooling.

[†]Comorbidities were assessed in the 3 years prior to study cohort entry, and medications and healthcare use were assessed in the year prior to study cohort entry.

[‡]In Saskatchewan, the number of non anti-diabetic drug classes was defined using the list of medication covariates rather than anatomical therapeutic chemical (ATC)-defined classes due to unavailability of ATC codes. In Quebec and the CPRD, drug classification was performed using the American Hospital Formulary Service and the British National Formulary respectively.

Table 2: Crude and adjusted hazard ratios for the association between the use SGLT2 inhibitors and the risk of below-knee amputation among patients with type 2 diabetes

Treatment group	No. of patients	No. of events	Mean follow-up time (years)	Person-years	Crude incidence rate (per 1,000 person-years)*	Crude HR (95% CI) †	Adjusted models‡	
							HR (95% CI)	I^2
SGLT2 inhibitors	207,817	253	0.90	187,641	1.3	0.87 (0.69-1.10)	0.88 (0.71-1.09)	18%
DPP-4 inhibitors	207,817	281	0.88	181,817	1.5	Reference	Reference	

Abbreviations: CI, confidence interval; DPP-4, dipeptidyl peptidase-4; HR, hazard ratio; SGLT2, sodium glucose cotransporter 2.
*Incidence rate was calculated using all study cohorts.
†SGLT2 inhibitors patients were matched to DPP-4 inhibitors patients from their exposure-set (defined on level of anti-diabetic therapy, time on DPP-4 inhibitors [for prevalent new users only], prior use of GLP-1 receptor agonists, and within 120 days of the SGLT2 inhibitor prescription) on time-conditional propensity score. HR estimation was restricted to sites with at least 5 events in each exposure groups.
‡Outcome models were adjusted for age (continuous), sex, diabetes duration (continuous) and deciles of time-conditional propensity score.

Table 3: Summary of results of stratified and sensitivity analyses of pooled adjusted hazard ratios (95% CI) for below-knee amputation for SGLT2 inhibitor use versus DPP-4 inhibitor use among patients with type 2 diabetes.

		Number of sites included	Adjusted HR (95% CI)*	I^2
Main analysis		**7**	**0.88 (0.71-1.09)**	**18%**
Age	≥70 years	3	1.13 (0.81-1.56)	0%
	<70 years	6	0.80 (0.58-1.12)	46%
Sex	Females	3	1.04 (0.64-1.70)	17%
	Males	6	0.87 (0.65-1.15)	38%
Prior insulin use†	Yes	6	0.69 (0.46-1.03)	51%
	No	4	1.14 (0.87-1.50)	0%
SGLT2 inhibitor molecule	Canagliflozin	5	0.98 (0.77-1.25)	0%
	Dapagliflozin	4	0.69 (0.45-1.06)	0%
	Empagliflozin	3	1.08 (0.70-1.68)	0%
Varying grace period	0 day	4	1.12 (0.74-1.69)	0%
	60 days	7	0.90 (0.73-1.12)	28%
	365 days	7	0.90 (0.72-1.11)	46%
New user status	Incident users	6	0.68 (0.53-0.88)	0%
	Prevalent users	3	1.29 (0.97-1.70)	0%
Intention-to-treat approach‡		6	0.81 (0.63-1.05)	35%
No prior history of dialysis		1	1.13 (0.81-1.57)	NA
Fixed-effects model analysis		7	0.90 (0.75-1.08)	18%

Abbreviations: CI, confidence interval; DPP-4, dipeptidyl peptidase-4; HR, hazard ratio; MACE, major adverse cardiac events; NA, non-applicable; SGLT2, sodium-glucose co-transporter 2

* Outcome models were adjusted for age (continuous), sex, diabetes duration (continuous) and deciles of time-conditional propensity score.

† Prior insulin use was defined as prescription for insulin in the year prior.

‡ In the intention to treat approach, maximum follow-up was 1 year.

Note: Inclusion in each meta-analysis was restricted to sites with at least 5 events in each exposure group.

Figure Legends

Figure 1: Flowchart describing construction of study cohort.

Figure 2: Hazard ratios (95% CI) of below-knee amputation associated with SGLT2 inhibitors use compared with DPP-4 inhibitors use among patients with type 2 diabetes*.