Metabolism Clinical and Experimental 96 (2019) 92–100

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Commentary

# Sodium-glucose co-transporter-2 inhibitors (SGLT2i) use and risk of amputation: an expert panel overview of the evidence☆

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ARTICLE INFO

*Article history:*
Received 21 January 2019
Accepted 6 April 2019

*Keywords:*
Sodium-glucose co-transporter-2 inhibitors
Empagliflozin
Dapagliflozin
Canagliflozin
Amputation
Cardiovascular risk
Renal outcomes

ABSTRACT

Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are oral antidiabetic agents that exert their glucose-lowering effect by increasing renal excretion of glucose. These drugs have been reported to beneficially affect cardiovascular (CV) and renal outcomes. However, concerns have recently been raised in relation to increased risk of lower-extremities amputation with canagliflozin and it remains unclear whether and to what extent this side effect could also occur with other SGLT2i.

The present expert panel overview focuses on the three SGLT2i available and widely used in the US and Europe, i.e. empagliflozin, canagliflozin and dapagliflozin and only refers briefly to other SGLT2i for which less data are available. The results of large CV outcome trials with these SGLT2i are presented, focusing specifically on the data in relation to amputation risk. The potential pathophysiological mechanisms involved in this side effect are discussed. Furthermore, available data reporting amputation cases in SGLT2i users are critically reviewed.

The expert panel concludes that, based on current data, increased amputation risk seems to be related only to canagliflozin, thus representing a drug-effect rather than a SGLT2i class-effect. The exact pathways underlying this drug-induced adverse event, possibly related to off-target drug effects rather than SGLT2 inhibition per se, should be elucidated in future studies. Continuous monitoring and pharmacovigilance is necessary and head to head trials would also be essential to provide definitive conclusions.

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## 1. Introduction

Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are oral antidiabetic drugs that lower plasma glucose levels via an insulin independent increase in renal glucose excretion [1,2]. Apart from glucose lowering, SGLT2i can beneficially affect several cardiometabolic factors, including body weight, abdominal obesity, blood pressure, atherogenic dyslipidemia, arterial stiffness and uric acid [3–6]. These drugs may also improve renal and cardiac function due to their effects on diuresis, sodium retention, oxidative stress, inflammation, glomerular filtration,

☆ The authors are members of a larger expert panel constituted to evaluate the effects of sodium-glucose co-transporter-2 inhibitors. None of the members receive any remuneration from the pharmaceutical industry or any other source for their participation and/or for writing this paper.

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https://doi.org/10.1016/j.metabol.2019.04.008
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adipokine production, $Na^+/H^+$ exchange, and myocardial "fuel" metabolism [7–12]. Restoration of diurnal metabolic rhythms is another mechanism of action of SGLT2i, potentially contributing to their cardiorenal benefits [13].

There are also data showing potential beneficial effects of SGLT2i on non-alcoholic fatty liver disease (NAFLD) [14–17], a hepatic manifestation of the metabolic syndrome that has been linked to type 2 diabetes mellitus (T2DM) development and increased cardiovascular (CV) as well as liver morbidity and mortality [18–21]. Furthermore, SGLT2i have been reported to decrease epicardial fat [22–26]. It should be noted that increased epicardial adiposity has been related to coronary artery disease, cardiac arrhythmias, CV risk factors, T2DM, NAFLD and chronic kidney disease [27–31]. In this context, excessive peri-organ or intra-organ adipose tissue, including epicardial, perivascular, peripancreatic, perirenal and intramuscular fat has been suggested as an underestimated CV risk factor [32].

### *1.1. SGLT2i: CV outcome and safety clinical trials*

Multiple risk factors should be targeted in T2DM patients to achieve CV disease prevention; SGLT2i may thus represent an ideal therapeutic option in such high-risk patients [33]. Furthermore, CV outcome trials have been conducted with SGLT2i, especially with empagliflozin, canagliflozin and dapagliflozin, evaluating the effects of these drugs on the risk of the composite endpoint of CV morbidity and mortality as well as heart failure (HF) hospitalization and renal outcomes [34,35].

Briefly, the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) enrolled patients ($n = 7020$, median follow-up = 3.1 years), with established CV disease (99% of the population). Empagliflozin significantly lowered the primary outcome of CV death, non-fatal myocardial infarction (MI) or stroke [hazard ratio (HR) 0.86; 95% confidence interval (CI): 0.74 to 0.99; $p = 0.04$ for superiority]; HF hospitalization rate [35% relative risk (RR) reduction; $p = 0.002$], CV death (38% RR reduction; $p < 0.001$) and all-cause mortality (32% RR reduction; $p < 0.001$) were also significantly reduced in empagliflozin-treated T2DM patients with established CV disease [36]. Furthermore, empagliflozin was associated with significantly lower rates of incident or worsening nephropathy (HR 0.61; 95%CI: 0.53 to 0.70; $p < 0.001$), doubling of serum creatinine levels (44% RR reduction) and renal-replacement therapy (55% RR reduction) compared with placebo [37].

With regard to side effects, empagliflozin-treated patients experienced significantly fewer adverse events (any, severe, serious or leading to drug discontinuation) and had significantly lower rates of acute renal failure or acute kidney injury compared with placebo [36]. However, urinary infections in women and genital infections in both genders were significantly more frequent in the empagliflozin groups than in the placebo group.

In the Canagliflozin Cardiovascular Assessment Study (CANVAS) Program ($n = 10{,}142$, mean follow-up = 188 weeks), canagliflozin significantly reduced the rates of the composite of non-fatal MI, non-fatal stroke or CV mortality (HR 0.86; 95%CI: 0.75 to 0.97; $p < 0.001$) in T2DM patients with established CV disease (65.6% of the total population) or with CV risk factors [38]. Furthermore, canagliflozin reduced the incidence of HF hospitalization (HR 0.67; 95%CI: 0.52 to 0.87). In canagliflozin-treated patients, progression of albuminuria (HR 0.73; 95%CI: 0.67 to 0.79) and the composite of the need for renal-replacement therapy, sustained 40% reduction in estimated glomerular filtration rate (eGFR) or renal-specific mortality (HR 0.60; 95%CI: 0.47 to 0.77) were also decreased. However, it should be noted that these renal benefits cannot be regarded as significant on the basis of the prespecified hypothesis testing sequence. All-cause and CV mortality were not significantly affected by canagliflozin [38].

In the CANVAS Program, canagliflozin use was associated with a doubled risk of amputations (HR 1.97; 95%CI: 1.41 to 2.75), primarily at the level of the toes or metatarsals (71% of cases) [38]; patients with a history of amputation or peripheral artery disease (PAD) had the highest absolute risk of amputation. Although the reported amputation cases were relatively rare both in the canagliflozin and the placebo group (6.3 vs 3.4 participants per 1000 patients-years, respectively), these numbers should be interpreted in the context of the numbers of patients using these drugs worldwide. Furthermore, amputations are not only linked to decreased quality of life but also to increased future limb-related events and CV morbidity and mortality [39–42]. Thus, these findings raised significant concerns on whether this adverse event represents a drug- or class-effect.

A post hoc analysis of the EMPAREG OUTCOME trial reported no increased risk of amputations with empagliflozin use [43] as did a previous pooled analysis of phase I-III empagliflozin clinical trials [44]. Furthermore, in an EMPA-REG OUTCOME subanalysis, empagliflozin did not increase the risk for amputations in T2DM patients with PAD at baseline [45].

With regard to dapagliflozin, a recent pooled analysis of phase I-III dapagliflozin clinical trials did not detect differences in the rates of side effects (including amputations) between dapagliflozin and placebo [46]. Furthermore, the results of its CV outcome trial, i.e. the Multicenter Trial to Evaluate the Effect of Dapagliflozin on the Incidence of Cardiovascular Events (DECLARE-TIMI58) have recently been published [47]. Briefly, 17,160 T2DM patients (6974 with established CV disease at baseline) were followed for a median of 4.2 years. Dapagliflozin did not significantly reduce the rate of the primary endpoint, i.e. CV death, ischemic stroke or MI (HR 0.93; 95%CI: 0.84 to 1.03; $p = 0.17$), but significantly lowered the rate of the co-primary endpoint, i.e. CV mortality or HF hospitalization (HR 0.83; 95%CI: 0.73 to 0.95; $p = 0.005$), compared with placebo in the overall study population [47]. The observed dapagliflozin-induced benefit in the co-primary endpoint was driven by decreases in the rate of HF hospitalization (HR 0.73; 95%CI: 0.61 to 0.88), whereas CV death was not significantly affected (HR 0.98; 95% CI: 0.82 to 1.17). In relation to secondary outcomes, all-cause mortality did not differ between the 2 groups, whereas renal events (defined as ≥40% reduction in eGFR to <60 ml/min/1.73m$^2$, new end-stage renal disease or renal death) were significantly reduced by dapagliflozin (HR 0.76; 95%CI: 0.67 to 0.87) [47]. However, it should be noted that since dapagliflozin did not affect the primary endpoint, the results for the secondary outcomes are only considered as hypothesis-generating. Subgroup analyses by enrollment stratum (i.e. established CV disease vs multiple risk factors) produced the same results.

In the DECLARE trial, genital infections and diabetic ketoacidosis were more frequently seen in the dapagliflozin group compared with placebo (HR 8.36; 95%CI: 4.19 to 16.68; $p < 0.001$ and HR 2.18; 95%CI: 1.10 to 4.30; $p = 0.02$, respectively), as were adverse events leading to drug discontinuation (HR 1.15; 95%CI: 1.03 to 1.28; $p = 0.01$) [47]. In contrast, the rates of major hypoglycemic events, acute kidney injury, bladder cancer and side effects considered as serious were significantly lower in the dapagliflozin group compared with placebo (HR 0.68; 95% CI: 0.49 to 0.95; $p = 0.02$, 0.69; 95%CI: 0.55 to 0.87; $p = 0.002$, 0.57; 95% CI: 0.35 to 0.93; $p = 0.02$ and 0.91; 95%CI: 0.87 to 0.96; $p < 0.001$, respectively). Amputation rate did not differ between the 2 groups (1.4% dapagliflozin and 1.3% placebo).

### *1.2. Pathophysiological mechanisms of the canagliflozin-induced risk of amputations*

The underlying mechanisms of canagliflozin-related risk of amputation are unknown [48]. In this context, the drivers of amputation in T2DM patients are multiple, complex and potentially synergistic such as chronic ischemia (due to micro- and macrovascular dysfunction), susceptibility to trauma (due to neuropathy), impaired wound healing and infection [49]. No study has provided evidence as to which pathways are affected by canagliflozin.

Volume depletion, induced by SGLT2i, along with decreased tissue perfusion in patients with impaired arteriolar reactivity, may promote

tissue necrosis and amputation [48]. However, diuresis and, subsequently, volume depletion is more evident at the early phase of SGLT2i treatment, whereas amputation occurred more frequently during the late phase of the CANVAS Program [50]. It should be noted that the duration of the EMPAREG OUTCOME and DECLARE trials was 3.1 and 4.2 years, respectively, whereas for CANVAS was 188 weeks (i.e. 3.6 years). It has also been suggested that if amputations occurred after canagliflozin discontinuation it could have been caused by deterioration of glycemic control and a reduction in hematocrit, subsequently worsening diabetic foot disease [50].

>10 years ago, a nested case-control study with 12,140 T2DM patients reported that patients on thiazide diuretics (alone or combined with other antihypertensive drugs) had a higher risk for lower-extremities amputations than those on angiotensin converting enzyme inhibitor monotherapy [odds ratio (OR) 6.11; 95%CI: 1.32 to 28.27] [51]. This drug-induced risk of amputations was increased with duration of drug use. The diuretic-induced effect could be attributed to hypovolemia and raised hematocrit. More recently, among T2DM patients from the SURDIAGENE French observational cohort ($n = 1459$, median follow-up = 7.2 years), diuretic users were significantly more prone to lower-extremity amputations than non-users (HR 2.08; 95%CI: 1.49 to 2.93; $p < 0.0001$) [52]. Further evidence is needed to elucidate the plausibility of such an effect. Of note, the percentage of patients on diuretics were 44.3, 43.2 and 40.6% in the CANVAS Program [38], the EMPAREG OUTCOME [36] and the DECLARE trial [47], respectively. Additionally, data on hematocrit levels were only reported in the EMPAREG OUTCOME trial [36] (approximately 4% increase compared with placebo) but not in the other 2 CV outcome trials.

Elevated triglycerides (TG) have been recognized as an independent risk factor for lower-extremity amputations [53–55]. Neither the CANVAS Program [38] nor the EMPAREG OUTCOME trial [36] reported the effects of canagliflozin and empagliflozin on TG levels. Of note, baseline TG concentrations in these trials were not excessive i.e. 177 mg/dl (2.0 mmol/l) in the CANVAS Program [38] and 168–173 mg/dl (1.90–1.95 mmol/l) in the EMPAREG OUTCOME trial [36], respectively. The DECLARE trial did not report TG levels [47]. However, canagliflozin, empagliflozin and dapagliflozin were shown to significantly decrease TG in T2DM patients in other studies or meta-analyses [56–62].

Of note, bilirubin may protect against the risk of amputation in T2DM patients [63]. A previous study showed that both canagliflozin and dapagliflozin caused small transient increases in total bilirubin levels in healthy individuals [64]. Canagliflozin was also reported to significantly increase bilirubin levels in T2DM patients [65], although conflicting results exist [66]. No changes in bilirubin levels were found in a previous pooled analysis of phase I-III empagliflozin clinical trials [44].

Smoking is a strong predictor of lower-extremity amputations [67]. Almost 18% of patients in the CANVAS Program were active smokers [38]; the EMPAREG OUTCOME trial [36] and the DECLARE trial [47] did not report the percentage of current smokers.

Whether SGLT2i can affect the function of small arteries remains to be elucidated. Of note, the percentage of patients with established CV disease at baseline was 99% in the EMPAREG OUTCOME trial vs 65.6% in the CANVAS Program and 40.6% in the DECLARE trial [36,38,47]. Diabetic microvascular complications were present at 30.7, 17.5 and 21% of the patients in the CANVAS Program for neuropathy, nephropathy and retinopathy, respectively, whereas a history of amputation was recorded in 2.3% of the patients [38]. No information on these baseline characteristics were reported in the EMPAREG OUTCOME [36] and the DECLARE trial [47].

Selectivity for SGLT2 over SGLT1 differs among SGLT2i, with empagliflozin showing the highest selectivity (>2500-fold), followed by dapagliflozin (>1200-fold) and canagliflozin (>250-fold) [68]. Furthermore, canagliflozin differs from empagliflozin and dapagliflozin since it can increase cellular AMP or ADP via activation of the AMPK (AMP-activated protein kinase) by Complex I of the respiratory chain inhibition [69]. SGLT2i may also differ in other molecular characteristics, such as lipophilicity; empagliflozin is the least lipophilic of the 3 SGLT2i, followed by dapagliflozin and canagliflozin with consequent reduced tissue/vascular penetration and different tissue distribution [https://easddistribute.m-anage.com/from.storage?image=ggFF1HvHCpILkpoFklm%2FrUsBaV%2FBJASLhoYF0MW0c4X8kY0iNnZLB3G9eoA97ukplyjVmhbcRdwiaBZSQMeTGQ%3D%3D]. Indeed, it has been reported that tissue/blood ratios were much higher for canagliflozin compared with dapagliflozin and empagliflozin in Sprague Dawley rats; empagliflozin was far less distributed into organs other than the kidneys than canagliflozin and dapagliflozin [https://easddistribute.m-anage.com/from.storage?image=ggFF1HvHCpILkpoFklm%2FrUsBaV%2FBJASLhoYF0MW0c4X8kY0iNnZLB3G9eoA97ukplyjVmhbcRdwiaBZSQMeTGQ%3D%3D]. Whether such variations play a role in the different risk for amputation seen between SGLT2i remains to be elucidated by future studies.

We could not find any evidence of regional difference in SGLT1/SGLT2 receptors in arteries of the lower extremities in humans. Whether such receptors are present in this location and play a role in the pathogenesis of amputations should be elucidated. In other words, highly-specific SGLT2i may not act in a potentially harmful way in these arteries. It should be noted that regional specificity in endothelial nitric oxide synthase), endothelin-1 and 5-hydroxytryptamine receptor distribution in the aorta, renal and femoral arteries was shown to differ in rabbits. Moreover, there were differences between healthy and diabetic rabbits [70]. Whether such regional distribution differences exist in humans remain to be established. In this context, SGLT2 is highly kidney specific, SGLT1 and SGLT4 are highly abundant in the skeletal muscles and the small intestine, whereas SGLT5 and SGLT6 are mainly expressed in the kidneys and the central nervous system, respectively [71,72].

The half-life of empagliflozin was estimated to be 12.4 h [73]; the corresponding values for dapagliflozin 10 mg and canagliflozin (100 and 300 mg) were 12.9, 10.6 and 13.1 h [74,75]. Due to the similarity in half-lives, this does not seem to be a key difference between these SGLT2i, at least in humans.

It should be noted that canagliflozin was the first SGLT2i approved by the US Food and Drug Administration (FDA) (in 29 March 2013) [https://www.accessdata.fda.gov/drugsatfda_docs/nda/2013/204042Orig1s000TOC.cfm], followed by dapagliflozin (in 8 January 2014) [https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2014/202293Orig1s000ltr.pdf] and empagliflozin (in 1 August 2014) [https://www.drugs.com/newdrugs/fda-approves-jardiance-empagliflozin-type-2-diabetes-4064.html]. In Europe, dapagliflozin was the first SGLT2i approved by the European Medicines Agency (EMA) (in 11 November 2012) [https://www.ema.europa.eu/en/medicines/human/EPAR/forxiga], followed by canagliflozin (15 November 2013) [https://www.ema.europa.eu/en/documents/overview/invokana-epar-summary-public_en.pdf] and empagliflozin (22 May 2014) [https://www.ema.europa.eu/en/medicines/human/EPAR/jardiance]. Therefore, there is a greater use of canagliflozin over the other 2 SGLT2i, at least in the US, providing more data on efficacy and safety. Furthermore, the probability of a treatment bias, i.e. avoidance of the use of empagliflozin and dapagliflozin (apart from canagliflozin) in T2DM patients at a high amputation risk, following the FDA announcements of this side effect, cannot be ruled out. However, in the RCTs mentioned above, increased amputation rate was reported only in relation to canagliflozin use (in the CANVAS Program) [38] and not to dapagliflozin (in the DECLARE trial) [47] or empagliflozin (in the EMPAREG OUTCOME trial) [36]. Similarly, pooled analyses of phase I-III empagliflozin and dapagliflozin clinical trials [44,45] did not detect differences in the rates of amputations between these 2 SGLT2i and placebo.

### *1.3. Warnings from health authorities in relation to the canagliflozin-induced risk of amputations*

FDA had already issued a warning for canagliflozin and the risk of amputations based on an interim analysis of the CANVAS program before the completion of the study [76] and issued a new safety alert for

canagliflozin and amputations following the publication of the CANVAS safety results [77]. The US FDA advises physicians to consider factors that may predispose patients to amputations (i.e. history of prior amputation, neuropathy, PAD and diabetic foot ulcers) before initiating canagliflozin [77]. Furthermore, patients on canagliflozin should be monitored for symptoms and signs in the legs such as pain, tenderness, sores, ulcers or infections [77]. If these complications occur, canagliflozin should be discontinued. It should be noted that the US FDA authorized a warning relating to amputation risk only for canagliflozin (and not for other SGLT2i).

The EMA Pharmacovigilance Risk Assessment Committee (PRAC) investigated this issue and decided to include a warning of a potential risk of toe amputation in the prescribing information for all SGLT2i available in the EU market (i.e. canagliflozin, empagliflozin and dapagliflozin) [78]. These recommendations were endorsed by the Committee for Medicinal Products for Human Use (CHMP), despite the fact that an increase in lower limb amputations has not been reported in studies with dapagliflozin and empagliflozin [78]. The EMA stated that lower limb amputation is an uncommon adverse event of canagliflozin (occurring in 1–10 patients in 1000) and recommended that physicians may consider canagliflozin discontinuation if patients develop significant foot complications such as skin ulcers or infection [78].

### *1.4. Updated literature on the association between SGLT2i use and the risk of amputation*

Since the publication of the CANVAS results, the association between the risk of amputations and SGLT2i use, whether drug- or class-specific, has gained increasing interest. Randomized controlled trials (RCTs), observational studies and pharmacovigilance reports may provide useful information. RCTs offer the highest level of evidence but there is a lack of head-to-head RCTs comparing different SGLT2i in relation to the risk of amputation [79]. Furthermore, incidents of amputation may not always be properly recorded in RCTs, thus underestimating their rate [79]. Observational trials and pharmacovigilance reports on SGLT2i-related risk of amputation have been recently published (see below). However, both observational studies and pharmacovigilance data, such as those released after the warnings by the US FDA and the EMA, are susceptible to bias, even when propensity score estimation and matching are performed to minimize this limitation (in observational studies) [79]. Furthermore, the main concern with all analyses is the low number of recorded amputations, creating a high level of uncertainty for any associations.

In a pharmacovigilance analysis using the US FDA Adverse Event Reporting System (FAERS), canagliflozin was related to a higher amputation risk compared with empagliflozin and dapagliflozin [80]. Briefly, 66 cases of SGLT2i-related amputations were recorded, 56 of which (86%) involved canagliflozin. Mean age of the patients was 60 years, mean treatment duration was 1.5 years and 86% of the patients were men [80]. The majority of these cases referred to toe amputation; there were 13 above-ankle leg or limb amputations, one hand amputation and two multiple amputations. In disproportionality analysis within the FAERS, the frequency of reports on amputation as a side effect was significantly greater in canagliflozin-treated patients (3.4 per 1000; 95%CI: 2.6 to 4.6) compared with those on non-SGLT2i drugs [proportional reporting ratio (PRR) 5.33; 95%: 4.04 to 7.04; $p < 0.001$], empagliflozin (PRR 2.37; 95%CI: 0.99 to 5.70; $p = 0.054$) or dapagliflozin (PRR 0.25; 95%CI: 0.03 to 1.76; $p = 0.163$) [80]. Interestingly, within the FAERS, canagliflozin-related amputations occurred more commonly in non-insulin treated patients. In contrast, insulin therapy was a significant predictor of amputation in univariate analysis (but not in multivariable adjusted analysis) in the CANVAS program [80]. However, data within the FAERS were insufficient to support a causal relationship between canagliflozin use and amputation.

A retrospective case-controlled study of T2DM patients with active diabetic foot wounds found no association between SGLT2i and the risk of amputation (OR 0.70; 95%CI: 0.29 to 1.71; $p = 0.43$) over a 30-month period [81]. Overall, 27 patients were on SGLT2i: 16 on dapagliflozin (59%), 9 on empagliflozin, 1 changed from dapagliflozin to empagliflozin and another from canagliflozin to dapagliflozin. Therefore, the results of this small study referring to dapagliflozin and empagliflozin (and not to canagliflozin), provide some reassurance on the use of these 2 SGLT2i in patients with open foot wounds in real world setting [81]. Furthermore, a recent meta-analysis of 14 RCTs found that SGLT2i, as a class, were not associated with amputation risk [82]. However, in subgroup analysis, patients on canagliflozin had an increased incidence of foot amputations compared with those on placebo or other oral antidiabetic drugs (OR 1.89, 95%CI: 1.37 to 2.60); this association was not observed in patients on empagliflozin (OR 1.02, 95% CI: 0.71 to 1.48).

A disproportionality analysis using the WHO global database of individual case safety reports (VigiBase) was recently performed; among 8,293,886 available reports (from January 2013 to December 2017), a positive signal for lower limb amputation with SGLT2i was reported (79 cases) [48]. The highest RR was found for canagliflozin [7.09 (5.25, 9.57)], followed by empagliflozin [4.96 (2.89, 8.50)] and dapagliflozin, for toe amputations only [2.62 (1.33, 5.14)] [48]. However, this analysis has some limitations including the limited number of reports and the high rate of missing data that led the authors not to take into consideration any previous history of amputation or CV disease, other comorbidities and concomitant drug therapy [48]. Furthermore, pharmacovigilance analyses are exposed to bias in relation to the disproportionality measure: media safety alerts, time since marketing or selective notification, as mentioned by the authors [48].

Another population-based cohort study in newly treated T2DM patients with established CV disease ($n = 25,258$; median follow-up = 1.6 years) from the US Department of Defense Military Health System (i.e. the Evidence for Cardiovascular Outcomes With Sodium Glucose Cotransporter 2 Inhibitors in the Real World study - EASEL) showed that use of SGLT2i was associated with an almost 2-fold increase in the risk of below-knee lower extremity amputation (HR, 1.99; 95%CI: 1.12 to 3.51) [83]. The majority of amputations were observed in canagliflozin-treated patients. After exclusion of patients with a history of amputation, the crude incidence rates for amputation were 0.19, 0.09, and 0.12 per 100 person-years for canagliflozin, dapagliflozin and empagliflozin, respectively, in the intention-to-treat cohort and 0.15, 0.10 and 0.16 per 100 person-years, respectively, in the on-treatment cohort [83]. It should be noted that the number of prescriptions of SGLT2i other than canagliflozin were limited, thus formal statistical comparisons were limited for evaluating the amputation risk between different SGLT2i, even with appropriate adjustments for confounding [83]. Furthermore, medication use relied on pharmaceutical dispensing records, thus misclassification of the exposure could have possibly occurred.

A US real-world retrospective cohort study (between April 2013 and October 2016) found no evidence of an elevated risk of below-knee amputation in canagliflozin-treated T2DM patients ($n = 73,024$) compared with non-SGLT2i users ($n = 226,623$) (HR 0.98; 95%CI: 0.68 to 1.41; $p = 0.92$) [84]. Similarly, the results of the OBSERVE-4D study (Canagliflozin vs Other Antihyperglycemic Agents on the Risk of Below-Knee Amputation for Patients with T2DM-A Real-World Analysis of >700,000 US Patients) that were recently published reported no increased risk for below-knee lower extremity amputation for canagliflozin (or other SGLT2i) users compared with non-SGLT2i users [85]. These findings were based on data from 4 large US administrative claims databases and were consistent in the subpopulation of patients with established CV disease [85]. It should be noted that the OBSERVE-4D study is a retrospective observational real-world trial, with associated strengths and limitations, and overall not permitting definitive conclusions. For example, as stated by the authors, the OBSERVE-4D study had a short follow-up time (median: 60–100 days on-treatment), thus having limited statistical power to detect differences in the 6 to 12-month period (the time at

which amputation risk started to emerge in the CANVAS Program) [85]. Of note, the US FDA reviewed multiple revisions of the protocol of the OBSERVE-4D study but without reaching final agreement prior to the initiation of the trial [86].

In another recent US retrospective cohort study (between September 2012 and September 2015), new users of SGLT2i (n = 39,869 patients) had a significantly higher risk for amputation compared with metformin, sulfonylureas and thiazolidinediones (adjusted HR 2.12; 95%CI: 1.19 to 3.77) but not compared with new users of glucagon-like peptide-1 (GLP-1) agonists (n = 39,120) (adjusted HR 1.47; 95% CI: 0.64 to 3.36) or dipeptidyl peptidase-4 inhibitors (DPP-4is) (n = 105,023) (adjusted HR 1.50; 95%CI: 0.85 to 2.67) [87]. Among patients on SGLT2i, the majority were on canagliflozin (n = 28,036), followed by those on dapagliflozin (n = 8647) and empagliflozin (n = 3186).

It should be noted that in this cohort study [87] the effects of different SGLT2i on amputation risk were not evaluated, since the sample size was insufficient to permit such analysis. Furthermore, they did not mention the EMPA-REG OUTCOME subanalysis on T2DM patients with PAD at baseline [45]. The duration of follow-up was relatively short (the cohort included new users of antidiabetic drugs between September 1, 2012 and September 30, 2015) and amputation rates were low [18, 41 and 11 cases for SGLT2i, DPP-4is and GLP-1 agonists, respectively] [87]. Furthermore, the percentage of patients with established CV disease was much lower in this study (≤2.3% cerebrovascular disease, ≤6.7% ischemic heart disease, ≤4.9% congestive heart failure) than in the CANVAS Program (65.6%) [38] the DECLARE trial (40.6%) and the EMPA-REG OUTCOME trial (99%) [36]. Similarly, statin use was extremely low (≤36.1%) in the Chang et al. study [87], whereas it was 74.7–75.2% in the CANVAS Program [38] and 76.0–77.9% in the EMPA-REG OUTCOME trial [36]. In the DECLARE trial [47] 74.9–75% of the patients were on statin or ezetimibe. Statins have been reported to significantly reduce the risk for lower-extremity amputations in T2DM patients with or without PAD [42,88–90]. All the above-mentioned differences do not permit safe conclusions and limit the clinical significance of the findings.

In a recent propensity-matched cohort study (conducted from July 2013 to December 2016) that involved nationwide registers from Sweden and Denmark, new users of SGLT2i (n = 17,213; 61, 38 and 1% on dapagliflozin, empagliflozin and canagliflozin, respectively) showed an increased risk of lower limb amputation (incidence rate 2.7 vs 1.1 events per 1000 person-years; HR 2.32; 95%CI:1.37 to 3.91) compared with new users of GLP-1 agonists (n = 17,213) [91]. This finding was independent of the history of PAD or lower limb amputation at baseline. However, the absolute risk difference [1.5 (95%CI: 0.4 to 3.3)] and the HR for toe or metatarsal amputation [1.55 (95%CI: 0.87 to 2.77)] were not significant [91]. Furthermore, this cohort study had several limitations, including the lack of separate analyses by different SGLT2i, the inability to control for low adherence and the presence of wide CIs due to the small number of events in each subgroup [91].

Another recent propensity-matched cohort study (n = 60,432 T2DM patients newly initiating either SGLT2i or DPP-4is, median follow-up = 0.6 years) reported 36 cases of amputations with SGLT2i and 24 with DPP-4i use showed a non-significant increased incidence of amputations with SGLT2i (HR 1.38; 95%CI: 0.83 to 2.31) [92]. The authors also mentioned that, in subgroup analyses, the amputation risk differed by SGLT2i type, being higher for dapagliflozin or empagliflozin (HR 2.25; 95%CI: 0.78 to 6.47), followed by canagliflozin (HR 1.15; 95% CI: 0.63 to 2.09) [92]. However, it should be noted that all reported results in this study were non-significant and that the majority of SGLT2i users (70%) were on canagliflozin, thus data on empagliflozin and dapagliflozin were substantially fewer than data on canagliflozin. Furthermore, the duration of follow-up (i.e. 0.6 years) was too short to allow any definitive conclusions in relation to drug-induced effects on amputation risk. Finally, there were several limitations, as stated by the authors, including lack of major clinical and sociodemographic data that could have affected the results. For all these reasons, these findings should be interpreted with caution.

The results of the randomized, double-blind, placebo-controlled CV outcomes trials with SGLT2i in relation to amputation rates are summarized in Table 1, whereas Table 2 presents the results of other studies/reports with SGLT2i in relation to amputation incidence.

SGLT2 inhibition has been associated with increases in plasma glucagon levels [93,94]. On the other hand, insulin and incretin-based therapies (i.e. DPP-4is and GLP-1 agonists) can suppress glucagon secretion [95], thus improving glycemia in SGLT2i-treated T2DM patients. To the best of our knowledge, whether the concomitant use of insulin,

**Table 1**
Results of the randomized, double-blind, placebo-controlled cardiovascular outcomes clinical trials with sodium-glucose co-transporter-2 inhibitors in relation to amputation rates.

| Clinical trial | Year of publication | n | Patient populations | Median follow-up | Treatment | Results in relation to amputations |
|---|---|---|---|---|---|---|
| EMPA-REG OUTCOME [36] | 2015 | 7020 | T2DM patients with established CVD | 3.1 years | Empagliflozin 10 or 25 mg vs placebo | In a *post-hoc* analysis of the EMPAREG OUTCOME trial, no increased risk of amputations was observed with empagliflozin [43]. Briefly, the incidence rate was 6.5 per 1000 patient-years in both groups (HR 1.00; 95% CI: 0.70 to 1.44).<br>Results were similar with empagliflozin 10 mg (HR 0.96; 95% CI: 0.63 to 1.47) and 25 mg (HR 1.04; 95% CI: 0.69 to 1.58).<br>Furthermore, in an EMPA-REG OUTCOME subanalysis, empagliflozin did not increase the risk for amputations even in T2DM patients with PAD at baseline [45]. Briefly, in patients with PAD at baseline, the rates of lower limb amputations were 5.5 and 6.3% in the empagliflozin and placebo groups, respectively (HR 0.84; 95% CI: 0.54 to 1.32); the corresponding rates for patients without PAD were 0.9 and 0.7%, respectively (HR 1.30; 95% CI: 0.69 to 2.46). |
| CANVAS Program [38] | 2017 | 10,142 | T2DM patients with established CVD (40.6%) or multiple CV risk factors (59.4%) | 188 weeks | Canagliflozin 100 or 300 mg vs placebo | Canagliflozin use was associated with a doubled risk of amputations (HR 1.97; 95%CI: 1.41 to 2.75; $p < 0.001$), primarily at the level of the toes or metatarsals (71% of cases).<br>The reported amputation cases were relatively rare both in the canagliflozin and the placebo group (6.3 vs 3.4 participants per 1000 patients-years; $p < 0.001$).<br>Patients with a history of amputation or PAD had the highest absolute risk of amputation. |
| DECLARE-TIMI 58 [47] | 2018 | 17,160 | T2DM patients with established CVD (65.6%) or multiple CV risk factors (34.4%) | 4.2 years | Dapagliflozin 10 mg vs placebo | Amputation rate did not differ between the 2 groups (1.4 vs 1.3% in the dapagliflozin vs placebo group; HR 1.09; 95%CI: 0.84 to 1.40; p = 0.53). |

EMPA-REG OUTCOME: Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME); CANVAS: Canagliflozin Cardiovascular Assessment Study; DECLARE: Multicenter Trial to Evaluate the Effect of Dapagliflozin on the Incidence of Cardiovascular Events; T2DM: type 2 diabetes mellitus; CVD: cardiovascular disease; CV: cardiovascular; PAD: peripheral artery disease; HR: hazard ratio; 95%CI: 95% confidence intervals.

**Table 2**
Results of other than randomized controlled clinical trials with sodium-glucose co-transporter-2 inhibitors in relation to amputation rates.

| Study | Year of publication | n | Patient populations | Mean follow-up | Treatment | Results in relation to amputations |
|---|---|---|---|---|---|---|
| Pharmacovigilance analysis (US FDA Adverse Event Reporting System) [80] | 2017 USA | 9,217,555 reported adverse events | N/A | 1.5 years | N/A | 66 cases of SGLT2i-related amputations were recorded, 56 of which (86%) involved canagliflozin. In disproportionality analysis, amputation rate was significantly greater in canagliflozin-treated patients (3.4 per 1000; 95%CI: 2.6 to 4.6) compared with those on non-SGLT2i drugs (PRR 5.33; 95%: 4.04 to 7.04; $p < 0.001$), empagliflozin (PRR 2.37; 95%CI: 0.99 to 5.70; $p = 0.054$) or dapagliflozin (PRR 0.25; 95%CI: 0.03 to 1.76; $p = 0.163$) |
| Retrospective case-controlled study [81] | 2018 Australia | 27 | T2DM patients with active diabetic foot wounds | 30 months | 16 patients were on dapagliflozin, 9 on empagliflozin, 1 changed from dapagliflozin to empagliflozin and another one from canagliflozin to dapagliflozin | No association between SGLT2i and the risk of amputation (OR 0.70; 95%CI: 0.29 to 1.71; $p = 0.43$) |
| Meta-analysis of 14 RCTs [82] | 2018 USA, Canada, Europe | 26,167 | N/A | 52 to 188.2 weeks | SGLT2i | SGLT2i as a class were not associated with amputation risk. However, in subgroup analysis, patients on canagliflozin had an increased incidence of foot amputations compared with those on placebo or other oral antidiabetic drugs (OR 1.89, 95% CI: 1.37 to 2.60); this association was not observed in patients on empagliflozin (OR 1.02, 95%CI: 0.71 to 1.48). |
| Disproportionality analysis using the WHO global database of individual case safety reports (VigiBase) [48] | 2018 Global | 8,293,886 reports | N/A | N/A | N/A | A positive signal for lower limb amputation with SGLT2i was reported (79 cases). The highest RR was found for canagliflozin [7.09 (5.25, 9.57)], followed by empagliflozin [4.96 (2.89, 8.50)] and dapagliflozin, for toe amputations only [2.62 (1.33, 5.14)] |
| Population-based cohort study [83] | 2018 USA | 25,258 | T2DM patients with established CVD newly treated | 1.6 years | N/A | SGLT2i use was associated with an almost 2-fold increase in the risk of below-knee lower extremity amputation (HR 1.99; 95%CI: 1.12 to 3.51). The majority of amputations were observed in canagliflozin-treated patients. After exclusion of patients with a history of amputation, the crude incidence rates for amputation were 0.19, 0.09, and 0.12 per 100 person-years for canagliflozin, dapagliflozin and empagliflozin, respectively. |
| Real-world retrospective cohort study [84] | 2018 USA | 346,190 | T2DM patients newly exposed to a SGLT2i (canagliflozin, dapagliflozin, or empagliflozin) or a non-SGLT2i antidiabetic drug (non-metf ormin) 22% of the patient population had established CVD at baseline | 0.43 years with canagliflozin and 0.33 years with non-SGLT2i therapy | 73,024 on canagliflozin 39,117 on dapagliflozin 24,433 on empagliflozin and 226,623 on non-SGLT2i antidiabetic drug | The crude incidence of BKLE amputation in the overall SGLT2i group was relatively low (1.22 per 1000 person-years), ranging from 0.96 to 1.26 and 1.39 per 1000 person-years with dapagliflozin, canagliflozin and empagliflozin, respectively. The corresponding rate for new users of non-SGLT2i drugs was 1.87 per 1000 person-years. In patients with established CVD the crude rate of BKLE amputation was 1.99, 1.28, 3.42, 2.03, and 3.29 per 1000person years with |

(continued on next page)

**Table 2** (*continued*)

| Study | Year of publication | n | Patient populations | Mean follow-up | Treatment | Results in relation to amputations |
|---|---|---|---|---|---|---|
| | | | | | | canagliflozin, dapagliflozin, empagliflozin, all SGLT2i, and non-SGLT2i antidiabetic drugs, respectively.<br>For patients without established CVD, the corresponding rates were 1.06, 0.88, 0.80, 1.00, and 1.05 with canagliflozin, dapagliflozin, empagliflozin, all SGLT2i, and non-SGLT2i antidiabetic drugs, respectively. |
| OBSERVE-4D study (Canagliflozin vs Other Antihyperglycemic Agents on the Risk of Below-Knee Amputation for Patients with T2DM-A Real-World Analysis of > 700,000 US Patients) [85] | 2018 USA | 714,582 | T2DM patients new users of SGLT2i or non-SGLT2i | 60–100 days on treatment | 142,800 new users of canagliflozin, 110,897 new users of other SGLT2i, 460,885 new users of non-SGLT2i | The risk for BKLE amputation with canagliflozin vs non-SGLT2i was 0.75 (95% CI: 0.40 to 1.41) in the on-treatment analysis and 1.01 (95% CI: 0.93 to 1.10) in the intent-to-treat analysis. |
| Retrospective cohort study [87] | 2018 USA | 953,906 | T2DM patients newly initiating SGLT2i or non-SGLT2i | 99–127 days on treatment | 28,036 on canagliflozin, 8647 on dapagliflozin, 3186 on empagliflozin, 105,023 on DPP-4i, 39,120 on GLP-1 agonists | New users of SGLT2i had a significantly higher risk for amputation compared with metformin, sulfonylureas and thiazolidinediones (adjusted HR 2.12; 95%CI: 1.19 to 3.77) but not compared with new users of GLP-1 agonists (adjusted HR 1.47; 95%CI: 0.64 to 3.36) or DPP-4i (adjusted HR 1.50; 95%CI: 0.85 to 2.67). |
| Propensity-matched cohort study [91] | 2018 Denmark | | T2DM patients newly initiating SGLT2i or GLP-1 agonists | From 270 to 274 days | 17,213 new users of SGLT2i (61% dapagliflozin, 61%, 38% empagliflozin, 38%, 1% canagliflozin) and 17,213 new users of GLP-1 agonists | There was an increased risk of lower limb amputation with SGLT2i (incidence rate 2.7 vs 1.1 events per 1000 person-years; HR 2.32; 95%CI: 1.37 to 3.91) compared with new users of GLP-1 agonists, independently of PAD or lower limb amputation history at baseline.<br>Of note, the absolute risk difference [1.5 (95%CI: 0.4 to 3.3)] and the HR for toe or metatarsal amputation [1.55 (95%CI: 0.87 to 2.77)] were not significant. |
| Propensity-matched cohort study [92] | 2018 USA | 60,432 | T2DM patients newly initiating either SGLT2i or DPP-4i | 0.6 years | 50% on SGLT2i and 50% on DPP-4i | There was a non-significantly differentincidence of amputations with SGLT2i (HR 1.38; 95%CI: 0.83 to 2.31). |

SGLT2i: sodium-glucose co-transporter-2 inhibitors; FDA: Food and Drug Administration; PRR: proportional reporting ratio; OR: odds ratio; HR: hazard ratio; RCTs: randomized controlled trials; GLP-1: glucagon-like peptide-1; DPP-4i: dipeptidyl peptidase-4 inhibitors; BKLE: below the knee lower extremity; N/A: not applicable.

DPP-4is or GLP-1 agonists can affect the increased amputation risk of canagliflozin has not been evaluated yet. However, as mentioned above, a pharmacovigilance analysis of the US FDA FAERS [80] showed that canagliflozin-related amputations occurred more commonly in non-insulin treated patients. Further research is needed to elucidate this issue. Furthermore, it remains unknown whether canagliflozin-induced amputation risk differs when canagliflozin is added as a second, third or fourth antidiabetic drug. A very recent publication identified the risk factors for canagliflozin-induced amputations in the CANVAS Program; these were: a prior amputation, PAD and neuropathy [96].

Regarding amputation risk with other SGLT2i, there are limited data in the literature. In this context, a post-marketing surveillance study with ipragliflozin (available only in Japan) found no increased risk of amputations [97]. This interim analysis involved 11,053 T2DM patients on ipragliflozin for 3 months, 5475 T2DM patients on ipragliflozin for 12 months and 138 T2DM patients ipragliflozin for 3 months. Similarly, tofogliflozin (also only available in Japan) did not cause amputations in a 52-week, multicentre, randomized, double-blind, open-label extension, Phase 4 study in Japan (J-STEP/INS) (n = 210 T2DM patients) [98]. Furthermore, ertugliflozin did not increase the risk of amputations in the VERTIS SITA phase III, randomized, double-blind, multicenter, placebo-controlled 26-week study (n = 291 T2DM patients) [99]. Further data (including larger numbers of patients and longer follow-up) are needed to elucidate whether these SGLT2i affect amputation incidence or not.

Overall, it should be noted that there are no head to head comparisons regarding amputations between different SGLT2i; this does not permit making any definitive conclusions. However, we need to consider the possibility that there may never be a properly designed and appropriately powered head to head comparison on this topic. Nevertheless, advice has to be based on currently available evidence i.e. avoiding canagliflozin should be considered, especially since there are evidence-based alternatives with no definitive signal for an increased risk of amputations.

In conclusion, although definitive studies are lacking and the topic is the focus of intense investigation, based on currently available data, SGLT2i-induced increased amputation risk seems to represent a drug-effect (related to canagliflozin) rather than a class-effect and the pathogenesis may be related to off-target effects rather than SGLT2 inhibition. Continued pharmacovigilance and head to head trials are essential to

provide definitive conclusions, but, unfortunately, well designed studies involving large enough numbers of patients may never be carried out. Further research (including involving other SGLT2i that have not yet been assessed in large event-based trials) is needed to identify the mechanisms underlying this drug-induced adverse event and to fully quantify risk associated with each SGLT2i.

## Declaration of interest

NK has given talks, attended conferences and participated in trials sponsored by Amgen, Angelini, Astra Zeneca, Boehringer Ingelheim, MSD, Mylan, Novartis, Novo Nordisk, Sanofi, Servier and WinMedica.

GD has received grants and personal fees (honoraria) from Abbott, Astra Zeneca, Boehringer Ingelheim, ELPEN, Galenica, MSD, Novartis, Novo Nordisk, Pharmaserv Lilly, Sanofi and VIANEX.

GH has received travel expenses, research grants, honoraria or advisory board fees from Medtronic, St. Jude Medical, Bayer, Novartis, Astra Zeneca, Boehringer Ingelheim, Pfizer, Sanofi, Vianex, ELPEN, Recordati, Servier and Pfizer.

NP has been an advisory board member of TrigoCare International, Abbott, AstraZeneca, Elpen, MSD, Novartis, Novo Nordisk, Sanofi-Aventis and Takeda; has participated in sponsored studies by Eli Lilly, Glaxo SmithKline, MSD, Novo Nordisk, Novartis and Sanofi-Aventis; received honoraria as a speaker for AstraZeneca, Boehringer Ingelheim, Eli Lilly, Elpen, Galenica, MSD, Mylan, Novartis, Novo Nordisk, Pfizer, Sanofi-Aventis, Takeda and Vianex; and attended conferences sponsored by TrigoCare International, AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novartis, Novo Nordisk, Pfizer and Sanofi-Aventis.

NT participated in advisory panels for Merck, AstraZeneca, Sanofi, Novo Nordisk, ELPEN, Eli Lilly and Company, Boehringer Ingelheim and Novartis and has received research support from Merck, Eli Lilly and Company, Novo Nordisk, Sanofi, Pfizer, Astra Zeneca, Janssen-Cilag, GlaxoSmithKline, and Novartis.

FT has received research support and honoraria from Amgen, Bayer, Boehringer Ingelheim, Elpen, Lilly, Menarini, Merck, Novartis, Sanofi, Servier, Vianex and WinMedica.

VT has given talks, attended conferences and participated in trials sponsored by Astra Zeneca, Boehringer Ingelheim, MSD, Eli-Lilly, Novartis, Novo Nordisk, Sanofi and Servier.

CT has received travel expenses or Research Grant or honoraria fees from the following: Medtronic, St. Jude Medical, Bayer, Novartis, Astra Zeneca, Boehringer Ingelheim, Pfizer, Chiesi, Pharmanel, Sanofi, Vianex, WinMedica, ELPEN, Recordati and Servier. CT is also the President of European Society of Hypertension and President of Hellenic Society of Cardiology.

DPM has given talks and attended conferences sponsored by MSD, AstraZeneca and Libytec.

CM has nothing to declare in relation to this paper and/or SGLT2i.

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