Universidade Federal do Rio Grande do Sul

Programa de Pós-Graduação em Ciências Médicas:

Endocrinologia

Safety and Efficacy of new antihyperglycemic agents in type 2 diabetes treatment: systematic reviews and meta-analyzes

Tese de Doutorado

Lana Catani Ferreira Pinto

Porto Alegre, 2019.

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Orientadora: Profa. Dra. Cristiane B. Leitão

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“Conhece-te,

Aceita-te,

Supera-te.”

(Santo Agostinho)

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SUMMARY

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Chapter 2

ABREVIATION'S LIST

UGDP - University Group Diabetes Program

FDA – Food and Drug Administration

EMA – European Medicines Agency

DPP-4 – Dipeptidyl Peptidase-4

GLP-1 – Glucagon-like peptide 1

SGLT2 – Sodium Glucose Transporter-2 inhibitors

CANVAS - Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes

EMPAREG - Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes

NNH – number needed to harm

CI – confidence interval

TSA – trial sequential analysis

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-analyses

PROSPERO - International Prospective Register of Systematic Reviews

RCT – randomised clinical trial

MEDLINE

EMBASE

MeSH - Medical Subject Heading

GRADE - Grading of Recommendations Assessment, Development and Evaluation

OAD – oral antidiabetics

NR – not reported

OR – odds ratio

HbA1c – glycated hemoglobin

RR – relative risk

RESUMO

A segurança dos novos agentes anti-hiperglicemiantes é uma causa de preocupação maior na prática clínica. Existem perguntas com relação à segurança pancreática das incretinas, tanto inibidores da DPP-4 quanto análogos do GLP-1. Por outro lado, os inibidores da SGLT-2 foram associados com efeitos adversos menores

como infecções genitais micóticas e infecções do trato urinário, mas também existem raros relatos de efeitos adversos mais graves. Como tanto análogos do GLP-1 quanto inibidores da SGLT-2 estão associados com redução da mortalidade em pacientes com diabetes melito tipo 2, o que provavelmente provocará aumento no seu uso no futuro, é muito importante definir seu perfil de segurança.

Outra pergunta não respondida com relação aos inibidores da SGLT-2 diz respeito aos benefícios clínicos das diferentes doses disponíveis dos agentes e a redução da HbA1c e do peso proporcionada por estas doses. Dado o exposto, os objetivos desta tese são: avaliar a segurança pancreática dos inibidores da DPP-4 com relação à pancreatite aguda e à neoplasia maligna de pâncreas; avaliar a segurança pancreática dos análogos do GLP-1 com relação ao câncer de pâncreas; avaliar os efeitos adversos associados aos inibidores da SGLT-2; e avaliar a eficácia das diferentes doses de inibidores da SGLT-2.

O primeiro estudo não achou associação entre inibidores da DPP-4 e câncer de pâncreas, no entanto, um pequeno risco para pancreatite aguda foi encontrado, apesar desse achado não ser definitivo.

O segundo estudo analisou a associação entre análogos do GLP-1 e câncer pancreático. Nesse estudo, o TSA confirmou que número suficiente de pacientes foi randomizado e que não há associação desse medicamento e câncer de pâncreas, considerando um NNH de 1000 e o tempo limitado de seguimento dos estudos incluídos (1,7 anos).

O último estudo explorou as diferenças entre os inibidores da SGLT-2 em doses diferentes e comparados um com o outro. Nessa análise, canagliflozina 300 mg pareceu o mais potente dos inibidores da SLGT-2 em reduzir a HbA1c e o peso, entretanto as diferenças não parecem ser clinicamente relevantes. Os demais inibidores da SGLT-2 em doses diferentes levaram a reduções similares em ambos os desfechos. Com relação aos

efeitos adversos, os inibidores da SGLT-2 foram associados com aumento no risco para infecções genitais.

Essa tese reafirma a segurança dos novos agentes anti-hiperglicemiantes. Os resultados também enfatizam a importância de prescrever os medicamentos anti-hiperglicemiantes considerando não apenas efeitos metabólicos e segurança, mas também eventos cardiovasculares e mortalidade.

ABSTRACT

The safety of new antihyperglycemic agents is a major source of concern in clinical practice. There are questions regarding pancreatic safety of incretins, either for DPP-4 inhibitor and GLP-1 agonists. On the other hand, SGLT-2 inhibitors have been

associated with minor side effects as genital and urinary infections but reports on rare and more serious outcomes have been published. As GLP-1 agonists and SGLT-2 inhibitors are associated with reduction in the mortality of type 2 diabetic patients, reason why its clinical use is expected to increase in the future, it is very important to clarify their safety profile.

Another unsolved question in SGLT-2 inhibitors is the clinical benefits of different commercially available agents and dosages on reduction of HbA1c and body weight. Given that, the objectives of these thesis were: to assess the pancreatic safety of DPP-4 inhibitors regarding acute pancreatitis and pancreatic cancer; to assess the pancreatic safety of GLP-1 inhibitors regarding pancreatic cancer; to assess the adverse events associated with SGLT-2 inhibitors; and to assess the efficacy of different doses of SGLT-2 inhibitors.

The first study didn't find an association between DPP-4 inhibitors and pancreatic cancer, however found a small risk for acute pancreatitis with DPP-4 inhibitors use, even though the latter finding is not definitive.

The second study analyzed the relationship between GLP-1 analogues and pancreatic cancer. In this study, TSA confirmed that enough patients were randomized and again no association of the medications and pancreatic cancer was observed considering a NNH of 1000 and the short mean follow-up of the included trials (1.7 years).

The last study explored the differences among SGLT-2 inhibitors in different doses and compared one to each other to one. In this analysis, canagliflozin 300 mg seemed to be the most potent SGLT-2 inhibitors in reducing HbA1c and body weight, however the differences don't look clinically relevant. The remaining SGLT2 inhibitors

in different doses lead to statistically similar effects for both outcomes. Regarding side effects, SGLT-2 inhibitors were associated with increased risk for genital infections.

This thesis reinforces the safety of the newest antihyperglycemic agents. The results also emphasize the importance of prescribing antihyperglycemic agents after considering not only metabolic effects and safety, but also cardiovascular events and mortality.

INTRODUCTION

Safety regarding new therapeutics has been a major concern in all areas of Medicine. In diabetes treatment, worries regarding medications' safety started in the very first randomised trial (1). In the University Group Diabetes Program (UGDP) study, patients randomised to tolbutamide were early discontinued due to excess of cardiovascular mortality (1). Moreover, patients randomised to phenformin experienced also greater cardiovascular mortality than insulin group. Importantly, more cases of lactic acidosis were reported with phenformin, including a fatal case (1). Later on, this adverse event lead to discontinuation of phenformin production and selling.

Decades later, troglitazone was approved by the Food and Drug Administration (FDA) in 1997, even though the medical officer assigned to evaluate the medication recommended against its approval(2). Soon after, reports of acute liver failure started showing up, and the manufacturer added warnings to the label of troglitazone, requiring monthly evaluation of liver enzymes (3). In the same year, it was removed from the market in England, later in the U.S.A and finally in Japan. It was never approved in the rest of Europe.

Other antihyperglycemic medications from the same class as troglitazone continued to be used for type 2 diabetes treatment for several years, albeit there was a concern of fluid retention with both rosiglitazone and pioglitazone. Rosiglitazone was commercialized in Europe until 2010, when a meta-analysis of randomised clinical trials suggested a higher risk of heart failure with rosiglitazone and two meta-analyses of cohort studies found a higher risk of heart failure compared to pioglitazone (4-6). In September 2010, the European Medicines Agency (EMA) recommended its suspension from the European market considering the benefits no longer outweighed the risks.

In reaction to the cardiovascular adverse events observed with glitazones, the FDA released a guideline containing rules for approval of new antihyperglycemic

agents(7). It stated that the trials should last longer than the typical 3 to 6 months and should contain the results of cardiovascular outcomes. In this way, the cardiovascular profile as well as adverse events would be further analyzed and described.

More recently, the focus of concerns with safety looked directly to incretins, both Dipeptidyl Peptidade-4 (DPP-4) inhibitors and Glucagon-LikePeptide-1 (GLP-1) agonists (8). In 2007, in response to reports of acute pancreatitis in patients using exenatide, the FDA added a warning on the medication labeling. In addition, concerns regarding thyroid safety were raised, as studies showed a small but increased risk of medullary thyroid cancer in rodents using liraglutide, one of the GLP-1 agonists (9; 10). Sitagliptin, a DPP-4 inhibitor, was also implicated with increased risk of acute pancreatitis (11). Several reports of pancreatitis, including fatal cases, have been described in people treated with sitagliptin and other DPP-4 inhibitors and some studies have assessed this association (12-14). Moreover, in 2016 the FDA released a new warning on DPP-4 inhibitors, regarding its association with heart failure (15).

Finally, the argument on side effects reached the youngest class of antihyperglycemic agents, the Sodium-Glucose Transporter 2 (SGLT2) inhibitors. Since its introduction, concerns on genital tract infections were raised (16). After that, some reports on the occurrence of ketoacidosis caused more rumors on the prescription of SGLT-2 inhibitors(17). Later, the results of the Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes (CANVAS) trial put canagliflozin on the spot leading diabetologists to ask if canagliflozin is related to an increase in limbs amputations (18). Finally, in September 2018 the rare occurrence of Fournier's gangrene was also reported in association with the use of this antihyperglycemic agent (19).

Yet on the SGLT2 inhibitors, some fase 2 and fase 3 studies had shown that different doses of SGLT-2 inhibitors produced similar changes in both HbA1c and body

weight (20; 21), but these studies had limited number of patients randomised and probably were overlooked. Nonetheless, the results of Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes (EMPAREG) outcomes trial were reassuring and deserve a second look. In this trial, the lower dose of empagliflozin (10 mg) produced the same reduction in A1c and cardiovascular outcomes as the higher dose (25 mg)(22). If lower doses produce lower incidence of adverse events is not clear with this trial.

All of these evidences show the importance of assessing the adverse effects of new medications and to counterpoise the potential risks and benefits when prescribing them .

Given the exposed above, the objectives of this thesis are:

- Assess the pancreatic safety of DPP-4 inhibitors regarding acute pancreatitis and pancreatic cancer.
- Assess the pancreatic safety of GLP-1 inhibitors regarding pancreatic cancer.
- Assess the adverse events associated with SGLT-2 inhibitors.
- Assess efficacy of different doses of SGLT-2 inhibitors.

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Chapter 2

Dipeptidyl Peptidase-4 inhibitors, pancreatic cancer and acute pancreatitis: A meta-analysis with trial sequential analysis

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ABSTRACT

The use of dipeptidyl peptidase-4 (DPP-4) inhibitors may be associated with pancreatic cancer and acute pancreatitis. Recent meta-analyses have reported conflicting findings. Therefore, we performed a meta-analysis to assess the risk of both pancreatic cancer and acute pancreatitis associated with the use of DPP-4 inhibitors. We also analysed whether the number of patients included is enough to reach conclusions, by means of trial sequential analysis. We included randomised controlled trials, lasting 24 weeks or more, that compared DPP-4 inhibitors versus placebo or other antihyperglycemic agents.

A total of 59,404 patients were included. There was no relationship between the use of DPP-4 inhibitors and pancreatic cancer (Peto odds ratio 0.65; 95% CI 0.35-1.21), and the optimal sample size was reached to determine a number needed to harm (NNH) of 1000 patients. DPP-4 inhibitors were associated with increased risk for acute pancreatitis (Peto odds ratio 1.72; 95% CI 1.18-2.53), with a NNH of 1066 patients, but the optimal sample size for this outcome was not reached.

In conclusion, there is no association between DPP-4 inhibitors and pancreatic cancer, and a small risk for acute pancreatitis was observed with DPP-4 inhibitors use, although the latter finding is not definitive.

INTRODUCTION

Dipeptidyl peptidase-4 (DPP-4) inhibitors or gliptins are incretin mimetic oral antihyperglycemic agents whose clinical use has steadily increased over the past ten years(1) . These medications are not associated with severe hypoglycemia and have a neutral effect on weight. However, there are concerns that the use of DPP-4 inhibitors may be associated with increased risk for pancreatic cancer and acute pancreatitis(2; 3) . An early study analysed the FDA reports of pancreatic cancer and concluded that there was a 2.7 fold increase in the risk for pancreatic cancer with DPP-4 inhibitor use(2) . Another study suggested that DPP-4 inhibitor use was associated with the occurrence of α-cell hyperplasia, that is, increased proliferation and dysplasia, with potential evolution into neuroendocrine tumors(4). Later, a pooled analysis of clinical trials with sitagliptin suggested no association between use of this medication and pancreatic cancer(5) . The lack of association between DPP-4 inhibitor use and pancreatic cancer was evaluated in a polled analysis including only two large randomised trials and no association was found(6) . Recently, three meta-analyses assessed the risk for acute pancreatitis among patients using gliptins. Li et. al. analysed the results of 60 randomised and non-randomised trials and found no increased risk of pancreatitis in patients treated with gliptins, compared to controls(7) . Despite this reassuring finding, the inclusion of observational studies might have influenced the results due to selection bias. Conversely, two other meta-analyses analysed the results of three large studies assessing the cardiovascular risk of sitagliptin, saxaglitin and alogliptin, and found contradictory results(1;3) In these studies, the use of DPP-4 inhibitors increased the risk of pancreatitis. Importantly, the potential cases of acute pancreatitis were adjudicated in these three trials.

Considering the potential association between DPP-4 inhibitor use and both pancreatic cancer and acute pancreatitis, we performed a meta-analysis including all randomised trials with DPP-4 inhibitor use of at least 24 weeks duration, in order to analyse whether there is an increased risk for pancreatic cancer and/or acute pancreatitis. We also assessed if the number of patients randomised in these trials is sufficient to reach definitive conclusions by means of trial sequential analysis (TSA).

METHODS

Protocol and registration

This systematic review and meta-analysis follows recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) protocol(8) and it was registered at the International Prospective Register of Systematic Reviews (PROSPERO) under the number CRD42016953346.

Patient Involvement

No patients were involved in the study.

Information source and search strategy

We performed a systematic literature search for all randomised clinical trials (RCTs) that compared DPP-4 inhibitor use with either placebo or other antihyperglycemic medications. We searched MEDLINE, EMBASE, Cochrane Central and Clinicaltrials.gov from database inception to May 2016. We also searched abstracts from the most recent meetings of the American Diabetes Association and the European Association for the Study of Diabetes. The search strategy combined the Medical Subject Heading (MeSH) terms “sitagliptin” OR “saxagliptin” OR “linagliptin” OR “alogliptin” OR “vildagliptin” AND “diabetes mellitus, type 2” AND a validated filter

to identify RCTs.(9) All eligible trials were considered for review, regardless of language. A manual search of reference lists of key articles was also performed.

Eligibility criteria

The inclusion criteria were: (1) RCTs, (2) DPP-4 inhibitor use versus any comparator, (3) treatment for at least 24 weeks, (4) definition of events of acute pancreatitis and/or pancreatic cancer, (5) inclusion of patients ≥18 y old, and (6) diagnosis of type 2 diabetes according to the American Diabetes Association criteria.(10)

Study selection and data collection

Two independent investigators (L.C.P. and S.S.B.) selected studies based on titles and abstracts. Studies satisfying inclusion criteria, or those with abstracts that lacked crucial information to decide upon their exclusion, were retrieved for full-text evaluation. Both investigators also analyzed the selected trials and extracted data; disagreements were resolved by a third reviewer (D.V.R.). The following information was extracted: first author's name, year of publication, sample size and dropouts, age, gender, trial duration, treatment in use prior to randomization, acute pancreatitis and pancreatic cancer events.

Risk of bias in individual studies and the quality of meta-analysis

The quality of studies was assessed according to the Cochrane Collaboration tool for risk of bias, including the six domains: random sequence generation; allocation concealment; blinding; incomplete outcome data; selective reporting; and other biases such as adjudication of events(11; 12). In adjudicated trails, the diagnosis was confirmed by the following criteria: symptoms of abdominal pain or vomiting and evidence of pancreatic inflammation (eg. elevated pancreatic enzymes, amylase or lipase > 3x the upper limit normal; in patients with chronic pancreatitis, enzyme elevations >2x the upper limit normal) or evidence of acute pancreatitis documented by imaging abdominal computerized tomography, magnetic resonance image or ultrasound

showing focal, diffuse and inhomogeneous gland enlargement. The quality of the metanalysis for each outcome (pancreatic cancer and acute pancreatitis) was evaluated by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (13). Each meta-analysis was rated as high, moderate, low or very low quality.

Synthesis of results

We compared the events of interest in patients randomised to use of DPP-4 inhibitors versus the events in patients randomised to the control strategy (placebo or other antihyperglycemic medications). The outcomes of interest were pancreatic cancer and acute pancreatitis.

Data was summarised with direct meta-analysis to compare DPP-4 inhibitors with placebo and other antihyperglicemic agents. We performed analysis through Peto odds ratio and Mantel-Haeszel. We used Peto odds ratio in the primary analyses as it is more conservative (can identify smaller associations), and is superior when dealing with rare events. Heterogeneity was assessed by the Cochran Q test (*p*-value of 0.1 was considered as statistically significant) and the I^2 test (values greater than 50% were considered to indicate elevated statistical heterogeneity). For studies with zero events in both arms, continuity correction was performed to include this data on TSA analyses. To access if the length of the trials was related to the outcome, we performed meta-regression, using study duration as a covariate.

Furthermore, to address whether current information is sufficient for firm conclusions, we performed TSA of the identified studies. This analysis is analogous to sample size estimation or interim analysis of a single study(14;15), and is associated with a cumulative meta-analysis which is represented by the Z-curve. Therefore, we calculated the sample size required to detect or reject a minimal relevant difference between DPP-

4 inhibitors and control(1; 4). We set this minimal relevant difference as an absolute difference of 0.1% in the incidence of both outcomes (pancreatic cancer and acute pancreatitis) between groups based on results of previous trials (1). We conducted the TSA with an overall 5% risk of type I error and 20% risk of type II error (power of 80%).

We evaluated publication bias with visual inspection of funnel plots and with Begg and Egger's tests. If a small study bias was identified, we would perform the trim and fill computation to explore the effect of missing studies on the outcomes.

The analyses were performed using RevMan software version 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and STATA 12.0 (Stata Inc., College Station, Texas, USA). The TSA was performed with TSA software (Centre for Clinical Intervention Research Department, Copenhagen, Denmark).

RESULTS

Our search retrieved 763 articles. After scanning through titles and abstracts, as well as removing duplicates, 186 articles remained for full-text evaluation. Afterwards, 38 trials were included for analysis (**Figure 1 – Study Flowchart**).

Selected studies were published between 2009 and 2015. Mean trial duration was 63.5 weeks (range, 24-260). The analysis included 59,404 patients, 39,970 (62.1%) were men and the mean age was 57.39 ± 5.12 years. The main characteristics of included trials are presented in **Table 1**. Results regarding the individual quality of included trials are presented in Supplemental Material.

The analysis of the funnel plots and Beggs and Egger's tests suggested no publication bias for either outcome (pancreatic cancer and acute pancreatitis).

DPP-4 inhibitors and pancreatic cancer

There were 16 events of pancreatic cancer in the DPP-4 inhibitor group and 24 events in the control group. DPP-4 inhibitors were not associated with increased risk for pancreatic cancer in the direct meta-analysis (Peto odds ratio 0.65; 95% CI 0.35-1.21) (**Figure 2A - Forest Plot for association between DPP-4 inhibitors and pancreatic cancer**). Similar results were observed with Mantel-Haenszel analysis (0.65; 95% CI 0.35-1.19). When we performed TSA, DPP-4 inhibitors were still not associated with pancreatic cancer (Peto odds ratio 0.66; 95% CI 0.36-1.19) and the number of randomised patients for this outcome surpassed the futility boundary (**Figure 2B - TSA for pancreatic cancer**). Meta-regression did not show an interference of study duration on the outcome (p = 0.867; 8 studies included) (**Supplemental Material**).

DPP-4 inhibitors and acute pancreatitis

There were 64 events of acute pancreatitis in the DPP-4 inhibitor group and 39 events in the control group. DPP-4 inhibitors were associated with an increased risk for acute pancreatitis in direct meta-analysis (Peto odds ratio 1.72; 95% CI 1.18-2.53; **Supplemental Material**) or with an absolute risk difference of 0.1% (representing a number needed to harm (NNH) of 1066). Mantel-Haenszel analysis showed comparable results (1.52; 95% CI 1.05-2.18). As we aimed to be conservative, TSA was performed to assess whether there was enough information to reach a definite conclusion regarding the association between DPP-4 inhibitors and acute pancreatitis. For this outcome, the number of patients evaluated (n = 59,404) did not reach the optimal sample size (n = 140,665) and the boundaries of benefit, harm or futility were not crossed, (Peto odds ratio 1.34; 95% CI 1.00-1.79). When performing meta-regression, no interference of study duration on acute pancreatitis was seen (p = 0.252; 25 studies included).

DISCUSSION

The results of the present review indicate that the use of DPP4 inhibitors is not associated with increased risk for pancreatic cancer. Furthermore, the TSA meta-analysis confirmed that the number of patients available was enough to reach this conclusion. There seems to be an association between the use of DPP-4 inhibitors and acute pancreatitis, even though the number of randomised patients for this conclusion was not enough, and the estimated risk for acute pancreatitis is small (one patient in 1066 patients treated with DPP-4 inhibitors).

Concern regarding the association between DPP-4 inhibitor use and pancreatic cancer was raised after a review of cases reported by the FDA(2). Other studies have suggested an association between DPP-4 inhibitor use and pancreatic cancer(4; 5) but there is still an ongoing debate on this topic. On the other hand, several observational studies have explored the association between DPP-4 inhibitors and pancreatitis(16; 17) However, due to study design characteristics, the results may be affected by selection and confounding biases. As there is a great number of randomised trials evaluating these medications, a systematic review with meta-analysis of these studies is recommended to properly address this clinical question.

Before this review, three other meta-analyses evaluated the association between clinical use of DPP-4 inhibitors and acute pancreatitis. The first one(7) did not find an association between use of DPP-4 inhibitors and acute pancreatitis; however, this review included not only randomised trials but also prospective and retrospective observational cohort studies. Most importantly, the events were not adjudicated. The other two(1; 3) found an increased risk of acute pancreatitis in patients treated with DPP-4 inhibitors; however, they only included the three large cardiovascular randomised trials, EXAMINE, SAVOR-TIMI 53 and TECOS(18-20) In these trials, a specialised committee adjudicated the diagnosis of acute pancreatitis. None of these

reviews performed TSA to evaluate whether the results were definitive and, more importantly, none of them evaluated the risk for pancreatic cancer associated with use of DPP-4 inhibitors.

Our study adds new information regarding this point. It included all randomised trials with DPP-4 inhibitor use that lasted for at least 24 weeks, and through TSA meta-analysis, evaluated whether the number of cases are enough to support the conclusions. There was a small risk for acute pancreatitis, so that it would be necessary to treat 1066 patients to have one case of acute pancreatitis, but the number of patients included in the meta-analysis was not sufficient to support this conclusion. Of note, due to the large number of diabetic patients using DPP-4 inhibitor worldwide, a great number of cases of acute pancreatitis might be prevented by taking into account pre-existing risk factors for acute pancreatitis, such as gallstones and hypertriglyceridemia, when considering prescription of this medication.

On the other hand, GLP1 agonist use is not associated with higher risk for acute pancreatitis, as recently pointed by a meta-analysis from Storgaard et al (21). Receptors for GLP-1 are largely found in the pancreatic ducts as well as in the pancreatic islets. Acinar and duct cells respond to GLP-1 therapy with proliferation(22; 23). A previous study in rats exposed to sitagliptin, reported hemorrhagic pancreatitis in one rat and acinar to ductal metaplasia in others(24). So, the association between incretins and acute pancreatitis has a biological plausibility. However, the explanation on why DPP-4 inhibitors are associated with pancreatitis and GLP-1 agonists are not, remains unclear(21).

When it comes to pancreatic cancer, no association between use of gliptins and pancreatic cancer was observed, and TSA meta-analysis showed that there were enough patients randomised for this observation.

The main limitation of our meta-analysis was the duration of the trials (mean of 63.5 minimum and maximum of 24 and 260 weeks) that may be insufficient to evaluate the development of pancreatic cancer. We tried to overcome this limitation including study duration as a covariate in the meta-regression and it did not have an influence on the outcome. However, we must consider that this analysis might have lack of power due to the number of included trials. The criteria used for diagnosis of acute pancreatitis in trials is a limitation. In adjudicated trails, the diagnosis was confirmed by an adjudication committee and the criteria used were clearly described. However, in non-adjudicated trials, the criteria used are not so straightforward. Nonetheless the analysis restricted to only studies with adjudication did not change the results. Furthermore, due to the design of the present study, we were not able to explore whether there is a specific sub-group of diabetic patients with increased susceptibility to this complication. The included trials did not describe the acute pancreatic risk factors, such as hypertriglyceridemia, alcohol consumption, and previous history of cholelithiasis. The only factor classically associated with acute pancreatitis that was mentioned was smoking status, and it was similar in intervention and controls groups.

Finally, there is enough information to suggest lack of association between the use of DPP-4 inhibitors and pancreatic cancer, but not of acute pancreatitis. The last one seems to be a continued concern and data of additional studies are needed. Despite this uncertainty, the implicated risk is small.

Author Contribution Statement. Author contributions: L.C.P. retrieved the full texts, abstracted the data, performed statistical analysis, wrote the first draft of the manuscript and revised the final version; D.V.R. retrieved the full texts, abstracted the data, and revised the final version of the manuscript; S.S.B. retrieved the full texts, abstracted the data and revised the final version of the manuscript; C.B.L. revised the final version of the manuscript; J.L.G. conceived the study idea and revised the final version of the manuscript. L.C.P. is the guarantor for the contents of the article, 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.

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Table 1. Characteristics of included trials

Author Year	***n***	**Follow-up (weeks)**	**Men (%)**	**Mean age (y)**	**Background treatment**
Ahren 2014	1012	104	47.6	54.4	Metformin
Araki 2013	561	26	70.4	60.0	Naïve or OADs
Arechevaleta 2011	1035	30	54.4	56.3	Metformin

Arjona-Ferreira 2013	426	54	59.8	64.2	Diet, Exercise or OAD
Bajaj 2014	272	24	48.5	53.8	Metformin + Pioglitazone
Barnett 2012	455	52	41.3	58.0	Insulin or Insulin + Metformin
Bergenstal 2010	514	26	51.7	52.5	Metformin
TECOS 2015	14671	260	70.7	66.0	Metformin, Pioglitazone, Sulphonylurea or Insulin
DeFronzo 2015	674	24	53.7	56.2	Metformin
DeFronzo 2012	743	26	46.4	54.1	Metformin
Del Prato 2014	2639	104	49.7	55.4	Metformin
Fredrich 2012	366	24	45.9	54.9	Naïve
Gallwitz 2012	1552	104	60.2	59.8	Metformin

Henry 2014	1615	54	56.5	NR	Diet, Exercise, Metformin or Sulphonylurea
Hollander 2009	565	24	49.6	54.0	Tiazolidinedione
Inagaki 2013	574	52	69.9	60.9	OADs
Jadzinsky 2009	1309	24	49.2	52.0	Naïve
SAVOR TIMI 53 2013	16492	140	66.9	65.0	Non-incretin therapies
Leiter 2014	507	52	53.7	63.3	OADs
Lewin 2015	667	24	53.8	54.6	Naïve
Mintz 2014	858	104	51.7	57.6	Metformin
Nauck 2007	1172	52	59.2	56.7	Metformin
Nauck 2014	1098	104	46.5	54.1	Metformin
Nowicki	170	52	42.9	66.5	OADs or Insulin

2011					
Olansky 2011	1250	44	56.8	49.7	Diet + Exercise
Pfutzner 2011	1306	76	49.2	52.0	Naïve
Pratley 2012	665	52	52.9	55.3	Metformin
Rosenstock 2009	401	24	50.9	53.5	Naïve
Rosenstock 2009	390	26	41.3	NR	Insulin
Rosenstock 2010	655	26	48.9	52.6	Naïve
Schernthaner 2013	756	52	55.9	56.7	Metformin + Sulphonylurea
Schernthaner 2015	720	52	61.8	72.6	Metformin
Seck 2010	1172	104	59.2	56.7	Metformin
Sheu 2015	1261	52	52.2	60.0	Insulin

Wainstein 2012	521	32	53.6	52.3	Diet + Exercise
EXAMINE 2013	5380	208	67.9	60.9	OADs
Weistock 2015	1098	26	47.4	54	Metformin
Williams-Herman 2012	306	24	52.0	53.7	Diet + Exercise

OADs oral antidiabetics; NR not reported

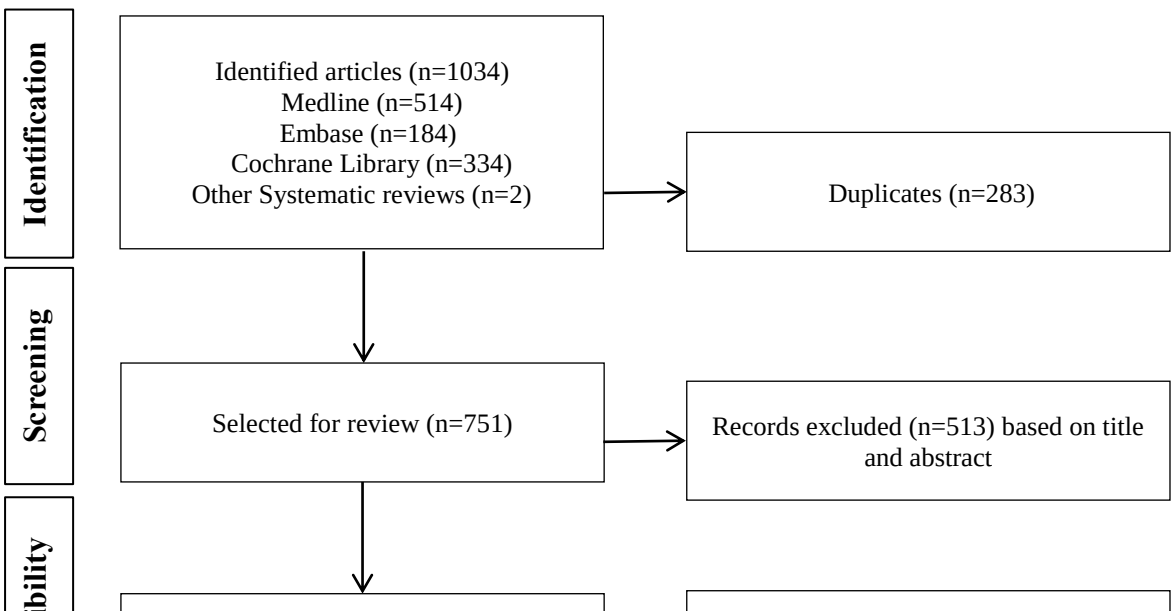


Figure 1 – Study Flowchart

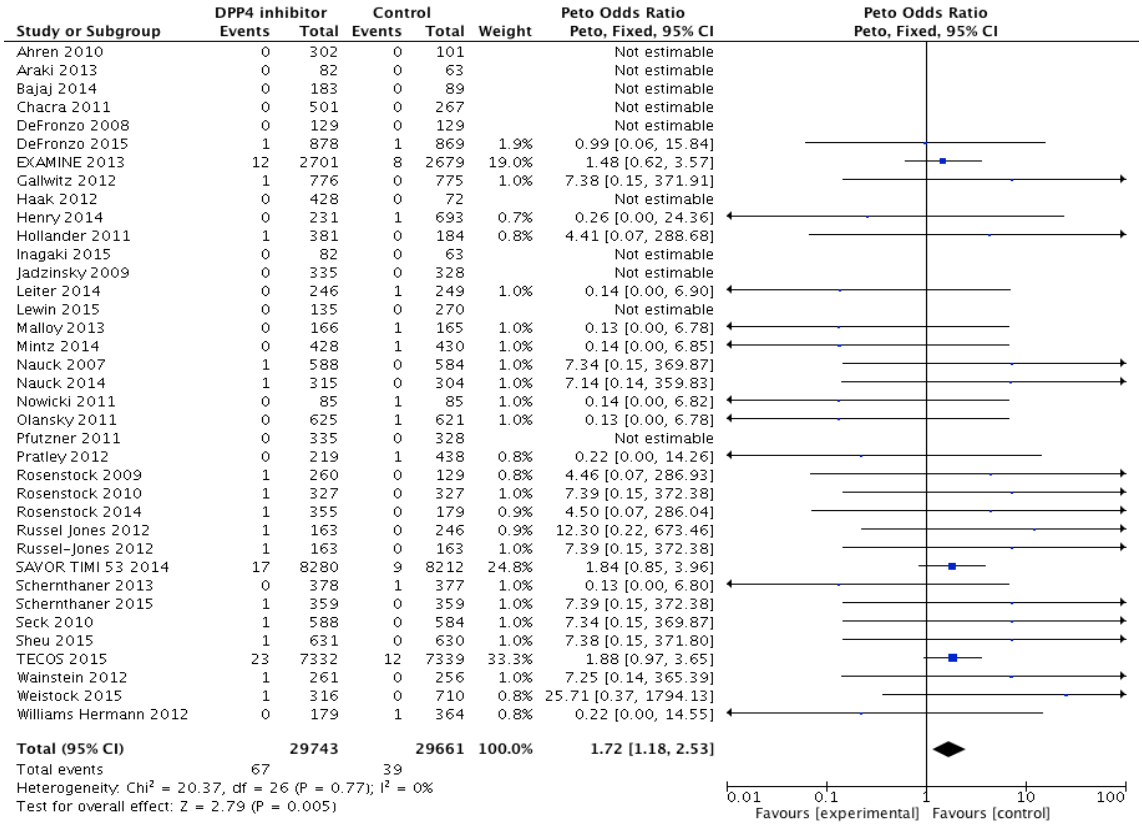


Figure 2A. Forest Plot for association between DPP-4 inhibitors and acute pancreatitis

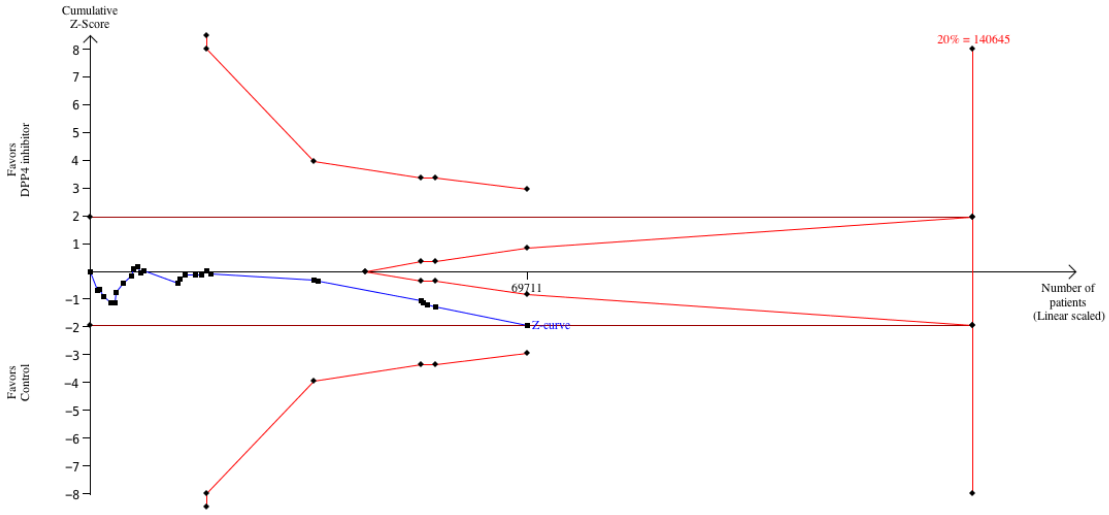
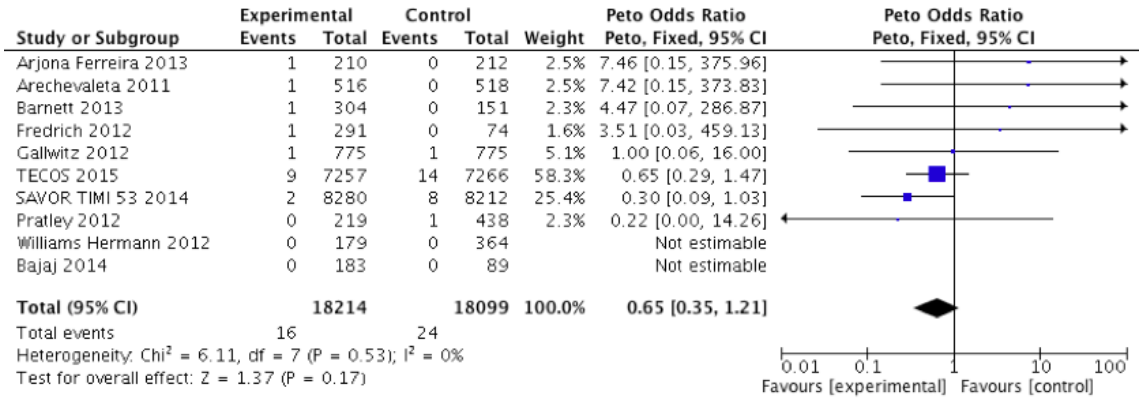
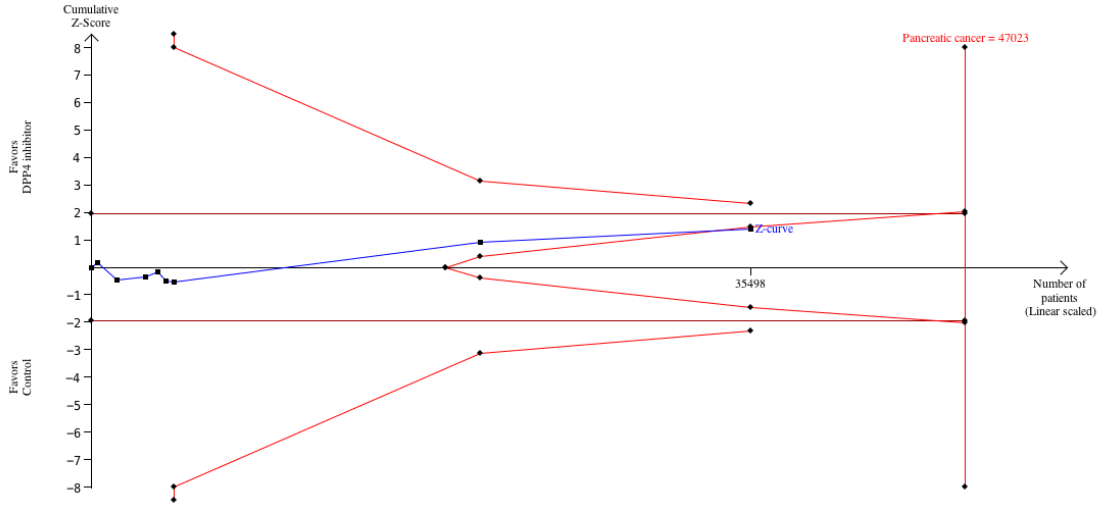


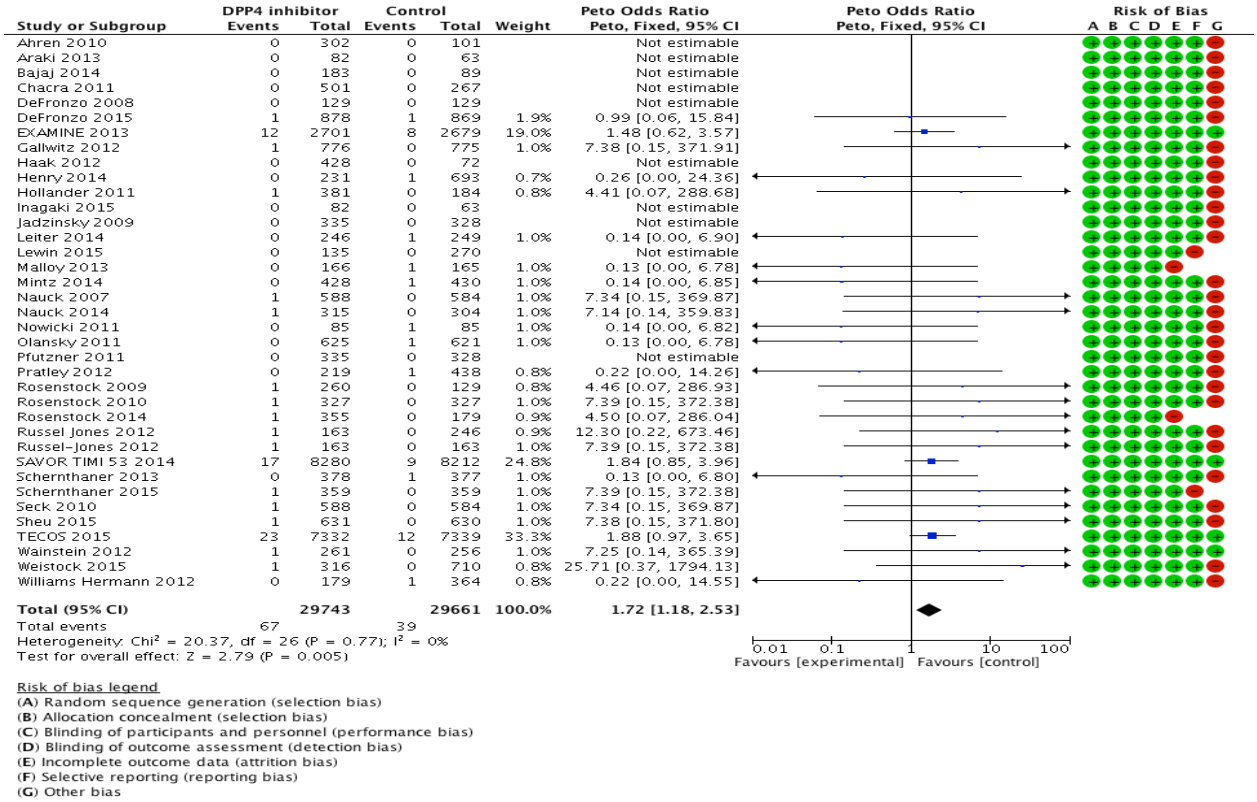
Figure 2B. TSA for acute pancreatitis



Supplemental figure 1. Forest Plot for association between DPP-4 inhibitors and pancreatic cancer



Supplemental figure 2. TSA for pancreatic cancer



Supplemental figure 3. Forest Plot for association between DPP-4 inhibitors and pancreatitis and risk of bias

Chapter 3

Glucagon-like peptide-1 receptor agonists and pancreatic cancer: a meta-analysis with trial sequential analysis

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ABSTRACT

We aimed to assess if GLP-1 agonists are associated with pancreatic cancer. Systematic review and meta-analysis of randomised trials with GLP-1 agonists as an intervention was performed. Trial sequential analysis (TSA) was performed to assess if the available information is sufficient to reject this association. Twelve trials met the study criteria, with a total of 36,397 patients. GLP-1 analogues did not increase the risk for pancreatic cancer when compared to other treatments (OR 1.06; 95% CI 0.67 to 1.67; I^2 14%). TSA confirmed that enough patients were randomised and again no association of the medications and pancreatic cancer was observed considering a NNH of 1000 and the short mean follow-up of the included trials (1.7 years). Larger studies with longer duration would be required to exclude a greater NNH and to aside concerns regarding possible influence of study duration and the outcome.

Keywords: GLP-1 agonist, meta-analysis, systematic review

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) agonists bind and activate the GLP-1 receptor, which results in lower glucose plasma values in diabetic subjects, increased satiety and reduced body weight. GLP-1 agonists promote the release of insulin in response to hyperglycaemia, inhibit the secretion of glucagon, slow gastric emptying, and augment satiety by directly affecting the central nervous system.(1) Receptors for GLP-1 are found in pancreatic islets and in pancreatic acini and ducts; basic research shows that GLP-1 therapy may lead to acinar and duct cell proliferation.(2; 3)

Based on observational data, a 2011 report identified an increased risk for pancreatitis and pancreatic cancer in patients on incretin therapy, (4) which led to a Food and Drug Administration (FDA) warning on the pancreatic safety of GLP-1 agonists.(5) Two short-term studies were performed at the FDA's request. These studies were carried out with exenatide and liraglutide in a rat model of diabetes, and they increased concerns with respect to the possible adverse effects of GLP-1 mimetic therapy on exocrine pancreas. Both drugs led to an elevation in pancreatic enzymes. One rat treated with exenatide died of pancreatic necrosis, and other animals had findings of acinar-to-ductal metaplasia and foci of ductal hyperplasia, which were interpreted as premalignant changes.(6; 7)

Later, a systematic review of case reports suggested that liraglutide therapy was associated with acute pancreatitis. (8) Nonetheless, a recent meta-analysis by Storgaard et al,(9) which included only trials with adjudicated pancreatitis events, did not show an association of GLP-1 agonists and acute pancreatitis.(9)

Notably, there still remains a controversy regarding pancreatic cancer. This topic was evaluated in two large cohort studies: in the first study, an increased risk for pancreatic

cancer was observed in “new users”,(10) whereas no relation was observed in the second study.(11) Another recent meta-analysis reported no association between GLP-1 agonist use and pancreatic cancer.(12) However, no attempt was made to ascertain if the available number of patients on GLP-1 agonist use or the number of events were enough for definitive conclusions. In the case of a meta-analysis with a negative result, it is crucial to establish if the pooled information is sufficiently powered to exclude the association between the factor being studied (GLP-1 agonist use) and the outcome (pancreatic cancer). Since the relation between GLP-1 agonists and pancreatic cancer is still unclear, the aim of this systematic review and meta-analysis is to assess if these anti-hyperglycaemic medications have an association with pancreatic cancer. We also aimed to determine if there is sufficient evidence to exclude this association by means of trial sequential analysis (TSA).

METHODS

Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) protocol recommendations’ were followed (13) and was registered at the International Prospective Register of Systematic Reviews (PROSPERO) under the number CRD42016953346.

We searched MEDLINE, EMBASE, Cochrane Central and Clinicaltrials.gov from database inception to September 2017. The search strategy combined the Medical Subject Heading (MeSH) terms “exenatide” OR “liraglutide” OR “semaglutide” OR “dulaglutide” OR “albiglutide” OR “lixizenatide”AND “diabetes mellitus, type 2” AND a filter to identify RCTs.(14) Regardless of language, all eligible trials were considered for review.

The inclusion criteria were as follows: (1) RCTs, (2) GLP-1 agonist use versus any comparator, (3) treatment for at least 48 weeks, (4) definition of events of pancreatic

cancer, (5) inclusion of patients ≥18 y old, and (6) diagnosis of type 2 diabetes according to the American Diabetes Association criteria.(15)

For trials that fulfilled all inclusion criteria but did not mention pancreatic cancer events, an e-mail was sent to the corresponding author asking for the data. Of 17 e-mails sent, four e-mails were returned to sender (the e-mail of the author did not exist or had changed) and 4 e-mails received replies, two of them containing pancreatic cancer data. Two independent investigators (L.C.P. and M.R.F.) selected studies based on titles and abstracts. Studies that met the inclusion criteria, or those with abstracts that lacked information to decide upon their exclusion, were included in full-text evaluation. Both investigators also analysed full texts and extracted data.

Risk of bias in individual studies and the quality of meta-analysis

In order to assess the quality of studies, the Cochrane Collaboration tool for risk of bias was used.(16) Regarding risk of bias, we considered the non-adjudication of events to be "other bias". Quality of meta-analysis was evaluated by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.(17)

Data Analysis

We compared the events of interest in patients randomised to use of GLP-1 agonists versus the events in patients randomised to the control strategy (placebo or other antihyperglycemic medications). The outcome of interest was pancreatic cancer.

Data were summarized with Mantel-Haenszel odds ratio (OR) with direct meta-analysis to compare the GLP-1 agonist group with the control group. Heterogeneity was assessed by the Cochran Q test (p-value of 0.1 was considered statistically significant) and the I^2 test (values greater than 50% were considered to indicate elevated statistical heterogeneity).

We performed a TSA on the identified studies to address whether the current evidence might be sufficient for firm conclusions. This analysis is associated with a cumulative meta-analysis represented by the Z-curve. Therefore, we were able to estimate the sample size required to accept or reject a minimal difference between GLP-1 agonists and control.(18; 19) This difference is arbitrary and must be clinically relevant. We set it as an absolute difference of 0.1% between groups, which is more conservative than the difference found in previous trials.(20) We conducted the TSA with an overall 5% risk of type I error and 20% risk of type II error (power of 80%). In this way, the analysis is able to reach a number needed to harm (NNH) of at least 1000. For studies with zero events in both arms, continuity correction was performed, and their data were included in TSA analyses.

Publication bias was evaluated with a visual inspection of funnel plots and with Begg's and Egger's tests. If a small study bias was identified, we then performed the trim and fill computation to explore the effect of missing studies on the outcomes.

The analyses were performed using RevMan software version 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and STATA 12.0 (Stata Inc., College Station, Texas, USA). The TSA was performed with TSA software (Centre for Clinical Intervention Research Department, Copenhagen, Denmark).

RESULTS

Our search retrieved 2099 articles. After running through titles and abstracts and removing duplicates, 48 articles remained for full-text evaluation. Finally, 12 trials were included for analysis (**figure 1**). In four of these trials, pancreatic cancer events were adjudicated.

Selected studies were published between 2011 and 2017. The mean trial duration was 1.74 years. The trials included 36,397 patients, 62.77% of whom were men and with a mean age of 58.0 ± 4.3 years. Characteristics of included trials are presented in **table 1.** The results regarding the individual quality of included trials are presented in Supplemental Material (**figure 1s**).

GLP-1 analogues did not increase the risk for pancreatic cancer when compared to the control OR 1.06; 95% CI 0.67 to 1.67; I^2 14%) (**figure 2**). When this analysis was repeated using only adjudicated trials, the results were left unchanged (OR 1.00; 95% CI 0.62 to 1.63; I^2 61%). TSA showed that the ideal sample size was 47,023, which was not reached (36,397). However, as the futility boundary was crossed, there is enough data to exclude the association between GLP-1 agonists treatment and pancreatic cancer (considering a difference of 0.1% between treatment groups) (**figure 3**). Considering results from all patients exposed to GLP-1 agonist use, the medication is safe, and a NNH as high as 1000 can be rejected. Funnel plot analysis did not show any small study bias (p=0.721).

The LEADER trial reported pancreatic cancer incidence in more than one way. The first approach used only the adjudicated cases (GLP-1 n = 13 and placebo n = 5). This analysis is depicted in figure 2. Their second approach identified four additional cases of death, which were attributed to malignancy related to pancreatic cancer (but without histological documentation) by the adjudication committee (GLP-1 n = 13 and placebo n = 9). Repeating the meta-analysis with this additional information did not change the results (OR 0.95 95% CI 0.61 to 1.48; I^2 0%).

To investigate if trial duration influences the outcome, we performed a meta-regression. No association was found (p =0.812; 95% CI -1.12 to 1.37) between trial duration and pancreatic cancer risk, but this analysis lacked power as only 7 studies were included.

DISCUSSION

Our findings reinforce that GLP-1 analogue use is not associated with pancreatic cancer. This conclusion is based on randomised studies with a mean follow-up of 1.74 years (minimum 1 year – maximum 3.5 years) and confirmation through TSA that enough patients have been studied so far to exclude this association for this length of time, long-term associations cannot be analysed with the current studies.

As our findings are based on good quality randomised trials, confounding and attrition bias are controlled, and the risk of unreliable results is diminished. Most importantly, our meta-analysis adds evidence to previous meta-analyses, (12; 18; 21) as it is the only one to incorporate the TSA approach, which allowed us to exclude a clinically relevant magnitude of the association between GLP-1 analogues and pancreatic cancer. In other words, we achieved a number needed to harm (NNH) as high as 1537 patients.

We must acknowledge that our findings are based on studies with different follow-up durations and pancreatic cancer definitions. We explored these limitations with meta-regression, as well as with subgroup analysis, and the results were unchanged. In addition, in a search of clinicaltrials.gov, 87 ongoing trials with GLP-1 analogues were found. To reach a higher NNH, the results of these trials will need to be taken into account. Another point to be considered is the 17 trials with unreported pancreatic cancer events, from which we only received replies of 4 authors.

Compared to previous studies, these results are reassuring. There have been concerns regarding the safety of GLP-1 agonists since Raufman et al. reported in the early 90s that GLP-1 interacted with exendin receptors on dispersed acini from guinea pig pancreas,(22; 23) and these concerns were increased with the results from the LEADER trial showing

a numerically greater, although statistically not significant, number of cases of pancreatic cancer in the liraglutide arm compared to the placebo.(20)

This study has some limitations. The follow up duration of the included trials may not be sufficient for the occurrence of carcinogenesis. We performed metarregression to evaluate if there was a trend towards higher incidence of pancreatic cancer in studies with longer duration, however no association between duration and the outcome was observed. Second, the limits of the confidence interval of the main outcome were 0.67 and 1.67, meaning that the real value of the statistics in 95% of the cases is somewhere in between those values, what indicates that besides the lack of association reported in this review, there is a chance that the medication could increase (as well as decrease) the risk of pancreatic cancer. In order to reassure our results, we performed direct meta-analysis and TSA, and in both cases the estimate was close to 1.0. Finally, it would be interesting to analyse the effect of each medication of the class separately, however due to the small number of studies with each one of them this analysis would lack power.

Our findings are relevant to patients with diabetes and obesity, as well as for physicians, as they reinforce the position of the European Medicines Agency, which considers incretins safe for use regarding pancreatic disease. In addition, other studies, most of which were observational, have shown similar results.(24; 25)

Ultimately, our analysis did not find an association of GLP-1 analogue use with pancreatic cancer in a mean follow-up of 1.74 years, and a sufficient number of patients have been randomised to be able to exclude a NNH of more than 1000 patients. To exclude smaller risks (i.e., a larger NNH) and to aside concerns regarding the influence of longer duration of medication exposition and the outcome, further evidence is needed.

Competing Interests: JLG reports grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), during the conduct of the study; the sponsor had no interference on data extracting, analyses and manuscript writing. LCP, DVR, SSB and CBL have declared that no competing interests exist.

Contribution Statement. L.C.P. retrieved the full texts, abstracted the data, performed statistical analysis, wrote the first draft of the manuscript and revised the final version; M. R. F. retrieved the full texts, abstracted the data and revised the final version; D.V.R. abstracted the data, and revised the final version of the manuscript; J. L. G. conceived the study idea, C.B.L. conceived the study idea and revised the final version of the manuscript. L.C.P. is the guarantor for the contents of the article, 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. **Funding:** This work was funded by the Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq) nº 307015/2010-6 and FIPE – Fundo de Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre.

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Table 1. Characteristics of Included Trials

Author, Year	**GLP-1 Agonist**	**Events Control**	**Events Intervention**	**Patients Control**	**Patients Intervention**
Marso, 2016 (SUSTAIN-6)	Semaglutide	4	1	1649	1648
Pfeffer, 2015 (ELIXA)	Lixizenatide	9	3	3034	3034
Marso, 2016 (LEADER)	Liraglutide	5	13	4672	4668
Holman, 2017 (EXSCEL)	Exenatide 1w*	15	16	7396	7356
Nauck, 2016	Dulaglitide	0	1	101	200
Kramer, 2015	Liraglutide	0	0	25	26
Home, 2015	Albiglutide	1	0	277	271
Diamant, 2014	Exenatide 1w*	0	0	233	223
Sathyanarayana, 2011	Exenatide 2d*	0	0	10	11

Pratley, 2011	Liraglutide	0	1	219	445
Bolli, 2014	Lixizenatide	0	1	160	322
Xu, 2014	Exenatide 2d*	0	0	274	142

*Exenatide 1w = exenatide once weekly; exenatide 2d = exenatide twice a day;

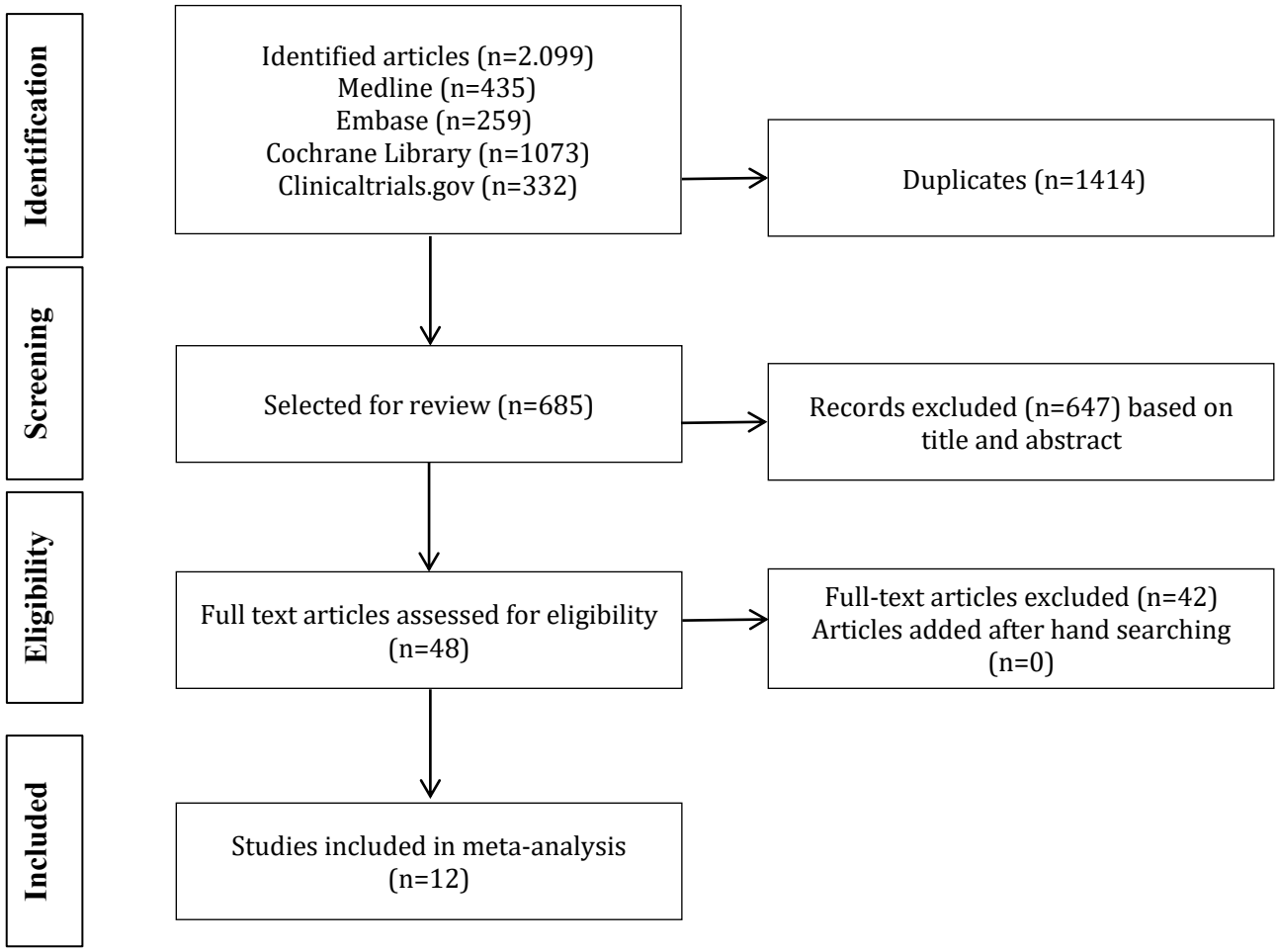

Figure 1. Study Flowchart

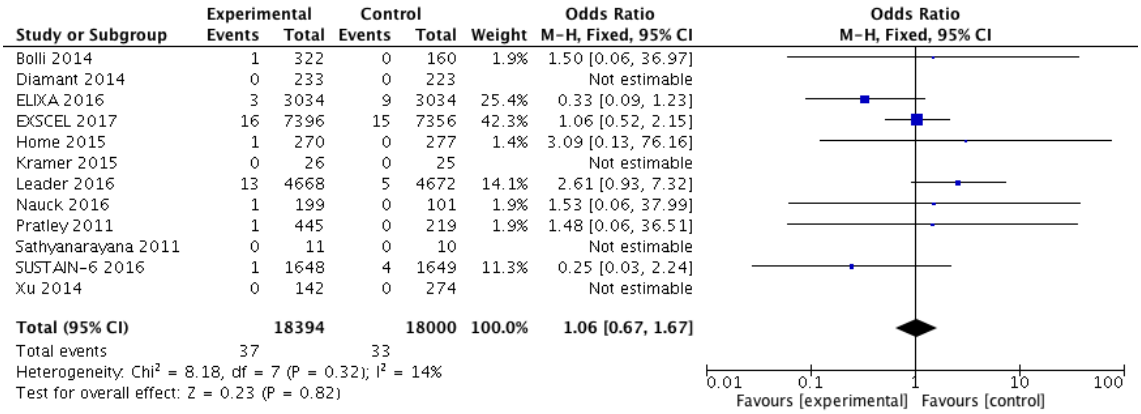

Figure 2. Forest Plot for association between GLP-1 inhibitors and pancreatic cancer

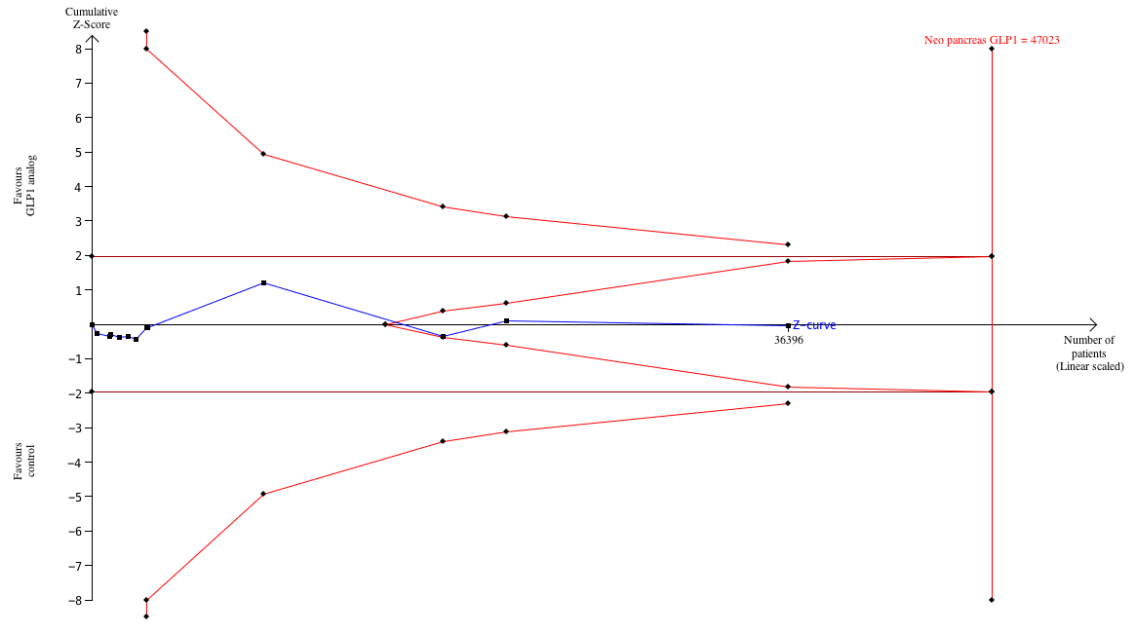

Figure 3. TSA for pancreatic cancer

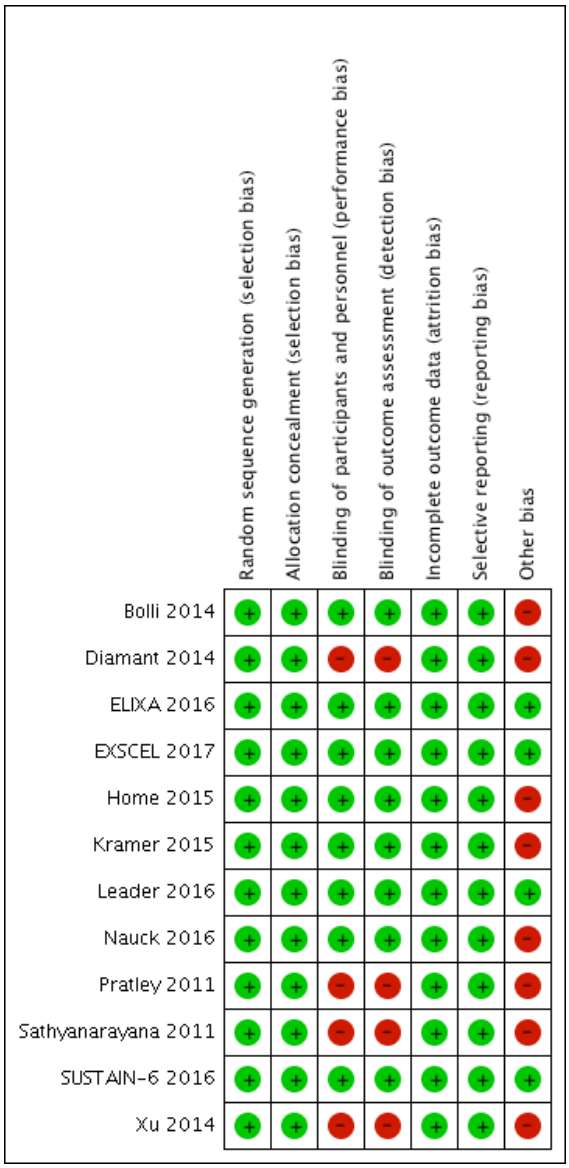

Figure 1S. Risk of bias

FINAL CONSIDERATIONS

This thesis reinforce the safety of the newest antihyperglycemic agents. The first study suggested an increased risk of pancreatitis with DPP-4 inhibitors use, however of small magnitude based on a large NNH. Regarding pancreatic cancer, the first two studies were able to exclude an association of DPP-4 inhibitors and GLP-1 analogues with the outcome, for at least a NNH of 1000 patients. For larges NNHs and guarantee of long-term safety, further studies are required.

The third study assured the safety of SGLT-2 inhibitors, as the only adverse event observed was genital mycotic infection. Notably, we showed that SGLT-2 inhibitors do not have a clinically significant dose range effect on HbA1c or body weight and these two variables should not be used as a guidance for increments in medications dosages of these particular agents.

Finally, besides the safety outcomes demonstrated here, benefits on cardiovascular events and mortality, such as those demonstrated on cardiovascular outcomes trials should be considered when selecting anti-hyperglycemic medications.