PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	**Metformin and pancreatic neuroendocrine tumors: a systematic review and meta-analysis**	1
ABSTRACT			
Structured summary	2	AIM The main aim of this review was to systematically analyze and summarize evidence related to the diagnostic and prognostic value of Type 2 Diabetes mellitus (T2DM) and metformin for predicting the insurgence and post-treatment outcomes of pancreatic neuroendocrine tumors (pNET). METHODS A systematic review of the published literature was undertaken, focusing on the role of T2DM and metformin in insurgence and prognosis of pNET, measured through outcomes of tumor-free survival, overall survival, and progression-free survival. RESULTS A total of 13 studies (n =5,674 patients) were included in this review. Analysis of 809 pNET cases from five retrospective studies (low study heterogeneity with I2=0%) confirms the correlation between T2DM and insurgence of pNET (OR=2.13, 95%CI=1.56-4.55; P<0.001). The pooled data from 1,174 pNET patients showed the correlation between T2DM and post-treatment tumor-free survival in pNET patients (HR=1.84, 95%CI=0.78-2.90; P<0.001). The study heterogeneity was intermediate, with I2=51%. A few studies limited the possibility of performing pooled analysis in the setting of metformin; therefore, results were heterogeneous, with no statistical relevance to the use of this drug in the diagnosis and prognosis of pNET. CONCLUSIONS T2DM represents a risk factor for the insurgence of pNET and is a significant predictor of poor post-treatment tumor-free survival of pNET patients. Unfortunately, a few studies with heterogeneous results limited the possibility of exploring the effect of metformin in the diagnosis and prognosis of pNET.	2,3
INTRODUCTION			
Rationale	3	Most patients with advanced pNETs die due to tumor progression. Therefore, identifying new therapies with low toxicity and good tolerability to use concomitantly with the established pNET treatment is relevant. In this perspective, metformin is emerging as a molecule of interest. Retrospective studies have described metformin, a widely used agent for the treatment of patients with type 2 diabetes mellitus (T2DM), to be effective in modulating different tumor-related events, including cancer incidence, recurrence, and survival by inhibiting mTOR phosphorylation. In pNET development, hyperactivation of PI3K/Akt signaling and activation of the mTOR pathway mediated through insulin-like growth factor-1 have been implicated to play a crucial role in carcinogenesis, thus providing the rationale for metformin use.	4,5

		Moreover, although risk factors for pNET are still inconclusive, T2DM has been described as an essential contributor to tumor development, with a high incidence and prevalence of diabetes seen among pNET patients. Indeed, the incidence of sporadic pNETs parallels that of T2DM, the highest in the fifth decade. Moreover, T2DM, through chronic hyperglycemia, might accelerate tumor cell growth and spread, a mechanism seen in many cancer types, which might also negatively affect pNET prognosis, while metformin in vitro leads to inhibition of NET cell aggressiveness.	
Objectives	4	The main goal of this review was to systematically analyze and summarize evidence relating to the diagnostic and prognostic value of T2DM and metformin for predicting the insurgence and post-treatment outcomes of pNET. Review questions: Q#1: Should type-2 diabetes mellitus impact the pNET insurgence or the survival outcomes in pNET patients? Q#2: Should metformin (or any other oral anti-diabetic strategy) impact the pNET insurgence or the survival outcomes in pNET patients?	5
METHODS			
Protocol and registration	5	Medline (PubMed) database was searched through June 2023 for relevant published original articles using the following keywords: (pancre* AND neuroendocrine tumor*) AND (diabetes OR T2DM OR mellit* OR MODY OR DM2) We also searched the reference lists of included studies. Two authors (QL and AC) independently reviewed the found records based on titles, abstracts, and the full text against the eligibility criteria	6
Eligibility criteria	6	This review focused on retrospective and prospective observational studies that evaluated the diagnosis and the post-treatment outcomes in pNET adults over 18 years. Studies were included if they investigated the diagnostic or prognostic value of T2DM or metformin measured in pNET patients. Case series, case reports, literature reviews, or studies without adequate prognostic analyses were excluded. Studies were selected based on the PICOTS framework. No geographic or follow-up restrictions were applied. Only studies in the English language were considered. A limitation in the year of publication was applied, excluding all the studies before January 2000. If a study featured multiple eligible articles, we chose the most recent paper with the most significant number of participants and the most extended duration of follow-up.	6
Information sources	7	Medline (PubMed) database	6
Search	8	Medline (PubMed) database was searched through June 2023 for relevant published original articles using the following keywords: (pancre* AND neuroendocrine tumor*) AND (diabetes OR T2DM OR mellit* OR MODY OR DM2) We also searched the reference lists of included studies	6
Study selection	9	Case series, case reports, literature reviews, or studies without adequate prognostic analyses were excluded. Studies were selected based on the PICOTS framework. No geographic or follow-up restrictions were applied. Only studies in the English language were considered. A limitation in the year of publication was applied, excluding all the studies before January 2000	6
Data collection process	10	Two independent reviewers (QL and AC) identified and collected data using the modified CHARMS-PF checklist[23]. Information extracted in each selected study included: First author (reference number), year of publication, country, period or study enrollment, design of the study, number of cases, number of controls, percentage of male sex, mean	6

		age, outcome measure, outcome value and 95% confidence intervals (95%CI).	
Data items	11	Two independent reviewers (QL and AC) identified and collected data using the modified CHARMS-PF checklist[23]. Information extracted in each selected study included: First author (reference number), year of publication, country, period or study enrollment, design of the study, number of cases, number of controls, percentage of male sex, mean age, outcome measure, outcome value and 95% confidence intervals (95%CI).	6
Risk of bias in individual studies	12	The Newcastle-Ottawa scale (NOS) was used to assess information on study quality; this scale varies from zero to a maximum possible score of nine and incorporates information on participant selection, outcome, exposure ascertainment, and the potential for confounding[24]. Two authors (QL and MC) assessed the included studies. Any discrepancies were resolved by consensus or by a third reviewer (VS).	7
Summary measures	13	Odds ratios (OR) or Hazard ratios (HR) with the corresponding 95%CI were used for the outcomes. Only the data adjusted for potential confounders were used to realize the pooled analyses reported in the present study. A random effects model was used to account for heterogeneity among studies. Heterogeneity was assessed using the Higgins I2 statistic[25]. An I2 >75% indicated high heterogeneity, 50–75% moderate heterogeneity, and <50% mild heterogeneity[26]. Forest plots were used to graphically display the effect size in each study and the pooled estimates. The heterogeneity of the different studies was graphically reported using the Galbraith plot and the Funnel plot. A P value <0.05 was considered statistically significant. All analyses were conducted using STATA statistical package version 14.0 (StataCorp LLC, College Station, TX, USA).	7
Synthesis of results	14	Heterogeneity was assessed using the Higgins I2 statistic[25]. An I2 >75% indicated high heterogeneity, 50–75% moderate heterogeneity, and <50% mild heterogeneity[26]. Forest plots were used to graphically display the effect size in each study and the pooled estimates. The heterogeneity of the different studies was graphically reported using the Galbraith plot and the Funnel plot. A P value <0.05 was considered statistically significant. All analyses were conducted using STATA statistical package version 14.0 (StataCorp LLC, College Station, TX, USA).	7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	The Newcastle-Ottawa scale (NOS) was used to assess information on study quality; this scale varies from zero to a maximum possible score of nine and incorporates information on participant selection, outcome, exposure ascertainment, and the potential for confounding[24]. Two authors (QL and MC) assessed the included studies. Any discrepancies were resolved by consensus or by a third reviewer (VS).	7
Additional analyses	16	The heterogeneity of the different studies was graphically reported using the Galbraith plot and the Funnel plot. A P value <0.05 was considered statistically significant. All analyses were conducted using STATA statistical package version 14.0 (StataCorp LLC, College Station, TX, USA).	7

RESULTS			
Study selection	17	The PRISMA flow diagram summarizes the study selection process (Figure 1). The search strategy identified 530 records and no records from reference lists. Records were screened based on the selection by title/abstract. Three hundred forty-seven records were excluded because they were irrelevant to the review question or did not adhere to the inclusion criteria. Of the remaining 183 eligible records, 170 full-text articles were discarded for several reasons (Figure 1). In detail, the reasons for discard were: non-human study (n=14), non-English (n=24), editorial/letter/case report/case series (n=103), no text available (n=2), review article (n=15), study not relevant (n=12). Key characteristics of the included studies are illustrated in Tables 1 and 2[27-39]. None of the studies included was a randomized controlled trial; only one was prospective, and the remaining 12 were retrospective experiences. No study reported was balanced after propensity score analysis.	7,8
Study characteristics	18	Studies were conducted between 2008 and 2022 in five countries: Italy (n=4), China (n=3), the USA (n=2), Germany (n=2), and France (n=1). One study was a European multicentric study. The study population ranged from 120 to 1,084 participants. The total number of cases enrolled was 5,674 cases. The mean patient age range was 54-62 years, and the percentage of males ranged from 40-56%. Heterogeneous outcomes were reported in the different studies. Five studies explored the role of T2DM as a risk factor for the insurgence of pNET[27-31], while the remaining eight studies explored the role of T2DM in terms of post-treatment outcomes[32-39]. The post-treatment outcomes were also heterogeneous, including progression-free survival (PFS), tumor-free survival (TFS), and overall survival (OS). As for the role of metformin, only five studies explored its role in the setting of pNET . In detail, two studies reported the role of metformin in the insurgence of pNET, and the remaining three explored PFS, TFS, or OS.	8
Risk of bias within studies	19	As reported in **Tables 1** and **2**, studies selected for review showed a good NOS, ranging from 6-9	8
Results of individual studies	20	Table 1.	18
Synthesis of results	21	In all the studies exploring the role of T2DM as a risk factor for pNET insurgence, this disease always resulted as a risk factor[27-31]. A meta-analysis was performed to explore this aspect. In patients with T2DM, the risk for pNET insurgence was significantly increased (OR=2.13, 95%CI=1.56-4.55; P<0.001). The heterogeneity of these studies was low, with an I2=0% (Figure 2A). The low heterogeneity was graphically observable, also looking at the Galbraith and Funnel plots (Figures 3A and 3B). Four studies explored the effect of T2DM in terms of post-treatment TFS [34-37]. The meta-analysis of HRs performed to explore this aspect showed that T2DM was a significant predictor of poor TFS (HR=1.84, 95%CI=0.78-2.90; P<0.001). The heterogeneity of these studies was intermediate, with an I2=51% (Figure 2B). The intermediate heterogeneity was also graphically observable in the Galbraith and Funnel plots (Figures 3C and 3D).	8,9
Risk of bias across studies	22	As reported in **Tables 1** and **2**, studies selected for review showed a good NOS, ranging from 6-9	8
Additional analysis	23	No additional anaylsis	
DISCUSSION			

Summary of evidence	24	Our results performed on 3,396 patients, including 809 pNET cases from five retrospective studies, confirm the correlation between T2DM and insurgence of pNET (OR=2.13, 95%CI=1.56-4.55; P<0.001)[27-31]. Possible mechanisms are still speculative and involve both chronic hyperglycemia, which is a hallmark of T2DM, and hyperinsulinemia. It seems that higher glucose availability to cancer cells, as present in T2DM, accelerates tumor growth, proliferation, and metastatic spread, while hyperinsulinemia might further promote tumor growth through direct and indirect effects. As a direct effect, insulin stimulates glucose uptake and consumption by the pNET cells, stimulating their proliferation, and indirectly, insulin displays mitogenic actions promoting cell division and spread and inhibiting apoptosis through the activation of the insulin receptor (IR)-IGF-1-receptor/phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mTORC1 pathway. Moreover, hyperinsulinemia downregulates the expression of IGF-1-BPs, which, in turn, enhances the bioavailability of IGF-1 and promotes its binding to IGF1R, leading to tumor cell growth[42]. In addition, low-grade chronic inflammation accompanying T2DM can also create a beneficial tumor microenvironment, promoting pNET growth and spread[43,44]. A few studies (mainly retrospective) have also reported the correlation between T2DM and the prognosis of pNETs. According to a study by Fan and coworkers, in the case of concomitant T2DM and pNET, patients had a greater chance for metastatic disease and neural invasion[36], greater tumor size[45], and poor survival post-pancreatic surgery[46]. We analyzed the pooled data from 1,174 pNET patients and found the correlation between T2DM and tumor-free survival in pNET patients (HR=1.84, 95%CI=0.78-2.90; P<0.001), suggesting higher recurrence risk in case of concomitant T2DM[32,33,34-39]. As T2DM seems to be a risk factor for contracting pNET and potentially negatively impacts the patients' outcomes, studies exploring the role of anti-diabetic agents, specifically metformin, in similar settings are of importance. Metformin has been investigated as an anticancer agent in the setting of different cancer types. In the case of pancreatic adenocarcinoma, its use in diabetic patients was associated with reduced cancer risk, while data on patients' survival are still inconclusive but also suggestive of positive effects[47]. The possibility to repurpose metformin in case of pNET treatment is suggested by the results of a small study by Pusceddu et al. where 12 patients with advanced G 1–2 pNETs and concomitant T2DM (compared to 19 patients without T2DM) had a significantly longer PFS if treated with metformin on top of everolimus 10 mg daily in combination with octreotide LAR 30mg i.m. every 28 days. Median PFS was 29 months in patients with T2DM taking metformin compared with 11 months in normoglycemic patients (p=0.018)[48]. A more extensive multicentric Italian study involving 445 patients with advanced pNETs suggests metformin, probably irrespective of its dose, significantly prolongs PFS of patients with T2DM compared to other anti-diabetic drugs used on top of everolimus with or without somatostatin analogs (44.2 months vs. 20.8 months), especially if introduced three months prior to standard anticancer treatment[32]. The *post hoc* analysis of the CLARINET study, including patients with advanced, non-functional entero-pancreatic NETs with an indolent course (both pNETs and intestinal NETS with a Ki67 ⩽ 10%) treated with lanreotide or placebo also showed a favorable effect of metformin on the PFS of patients who had T2DM prior to study treatment and were randomized to the placebo arm. In this patient subgroup, PFS more than doubled compared to patients not receiving metformin. On the other hand, there was no additional benefit when	9-11

		metformin was added to patients treated with lanreotide[33].	
Limitations	25	The present study presents some limitations. Only one prospective study was available, and no RCT was present among the investigable studies. Therefore, heterogeneity across the studies and potential inclusion biases should be considered. Second, it was impossible to perform detailed pooled analyses concerning several outcomes due to the paucity of studies to consider. This limitation was particularly true in the case of metformin studies. Lastly, several potential confounders that are impossible to analyze should be considered, like the duration of T2DM, the concomitant use of insulin, or the duration of anti-diabetic therapies. This type of data should be relevant in constructing meta-regressions, but unfortunately, these data were missing in several explored studies.	11,12
Conclusions	26	In conclusion, until results of RCTs, including patients with pNETs with or without concomitant T2DM receiving metformin in different proven anticancer treatments, become available, data on metformin effects in this setting is still inconclusive.	12
FUNDING			
Funding	27	No funding was provided	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097