REVIEW

Pioglitazone use in patients with diabetes and risk of bladder cancer: a systematic review and meta-analysis

Huaqing Yan
Haiyun Xie
Yufan Ying
Jiangfeng Li
Xiao Wang
Xin Xu
Xiangyi Zheng

Department of Urology, First Affiliated Hospital, Zhejiang University, Hangzhou, Zhejiang Province, China

Correspondence: Xiangyi Zheng
Department of Urology, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China
Email zheng_xy@zju.edu.cn

Abstract: Pioglitazone has been reported to increase the risk of bladder cancer but the conclusions of published clinical studies are confusing. We conducted a systematic review and meta-analysis of all eligible randomized controlled trial (RCT) studies and observational studies, in order to identify a more precise relationship between pioglitazone and risk of bladder cancer. We searched for publications up to January 24, 2018, in PubMed, EMBASE, Scopus, Web of Science, Cochrane register, and Chinese National Knowledge Infrastructure databases, and the references of the retrieved articles and relevant reviews were also checked. Relative risk and 95% confidence interval (CI) were used to assess this correlation. A dose-related meta-analysis was performed as well. Data on RCT studies showed a null association between pioglitazone and bladder cancer. The pooled RR estimates of the 12 included studies illustrated that pioglitazone is associated with a 14% increased risk of bladder cancer (95% CI 1.03–1.26). No evidence of publication bias was detected. In the dose effect analysis, patients who used a higher dose of pioglitazone had an increased risk of bladder cancer. In conclusion, this meta-analysis indicated that pioglitazone is associated with an increased risk of bladder cancer. Further research should be conducted to confirm our findings and reveal the potential biological mechanisms.

Keywords: thiazolidinedione, bladder tumor, epidemiology, dose effect, risk factor

Introduction

Bladder cancer has become a common cancer worldwide and ranks as the ninth most frequently diagnosed cancer. In 2012, approximately 430000 new cases of bladder cancer were diagnosed.[1] Cigarette smoking, specific exposure to arylamine, and chronic schistosoma infection are the most associated factors with increased risk for bladder cancer.[2] In recent years, diabetes mellitus (DM) has been reported to increase the risk of bladder cancer.[3,4] Meanwhile, pioglitazone, an antidiabetic agent of the thiazolidinedione (TZD) class, which is broadly used for glycemic control in patients with type 2 diabetes mellitus (T2DM), has been reported to increase the risk of bladder cancer as well. At first, the risk was reported in male rats,[5] then, a series of clinical studies was performed to identify this relationship. However, the conclusions of published observational clinical studies remain confusing.[6–25] This may result from problems associated with study design, limited sample size, and a lack of attention to potential biases. In 2016, one randomized controlled trial (RCT) (PROactive) ended its 10-year observational follow-up drawing a conclusion that the imbalance in bladder cancer cases observed during the double-blind period did not persist, and there was no overall increase in malignancies.[26] But only one RCT result could not end the argument. In 2017, another

RCT (TOSCA.IT) also reported null association between pioglitazone and bladder cancer, but the number of bladder cancer cases in this RCT was only 16, which may not be convincing.[27] A large cohort study including 193099 people by Lewis et al also reported no statistically significant increased risk of bladder cancer associated with pioglitazone use.[8] However, the updated US Food and Drug Administration review in 2016 concluded that use of T2DM medicine pioglitazone may be linked to an increased risk of bladder cancer,[28] which conflicted with the newly updated RCT results by Erdmann et al,[26] Vaccaro et al,[27] and cohort study by Lewis et al.[8] In the most recently updated meta-analysis conducted by Davidson and Pan,[29] they suggested the resurrection of the use of pioglitazone, but failed to perform sensitivity analysis and simply pooled observational studies together without excluding the duplicate population and low-quality studies (Table S1).[16,17,20–22] In another recent meta-analysis by Filipova et al,[30] they failed to give any details about quality assessment and simply pooled RCT and observational studies together without excluding the duplicate population and low-quality studies. They did not perform subgroup analysis either, though his result showed significant heterogeneity. Other meta-analyses are not convincing mainly because inadequate evidence acquisition, poor methodology, and lack of appropriate quality assessment. Consequently, despite a number of observational studies and previous reviews,[29,31–33] whether pioglitazone increases the risk of bladder cancer or not still remains an enigma. We conducted a systematic review and meta-analysis of all eligible RCTs, cohort studies, and nested case–control studies about pioglitazone use in patients with diabetes and bladder cancer in order to identify a more precise and reliable relationship between pioglitazone and risk of bladder cancer. Furthermore, we also examined whether the association between them converts according to sensitivity analysis.

Materials and methods
Publication search
This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[34] We carried out a search in PubMed, EMBASE, Scopus, Web of Science, Cochrane register, and Chinese National Knowledge Infrastructure databases, covering all the eligible papers published up to January 24, 2018. The search strategy included terms for outcome (bladder neoplasm* or bladder cancer* or bladder tumor* or bladder tumour*) and exposure (pioglitazone* or TZD* or thiazolidinedione*). We carefully evaluated every potentially relevant publication by examining their titles and abstracts.

All the studies matching the inclusion criteria were retrieved. The references from retrieved articles and reviews were also thoroughly checked to identify any additional relevant studies. All analyses were based on previous published studies, thus no ethical approval and patient consent are required.

Inclusion criteria
Studies included in this meta-analysis had to meet all the following criteria: a) an RCT design or a cohort design or a nested case–control design; b) the population included in studies were patients with diabetes; c) one of the exposures of interest was pioglitazone; d) one of the outcomes of interest was incidence of bladder cancer and the result is recorded in a reliable database or using ICD codes of cancer; e) studies provided rate ratio, hazard ratio (HR) or standardized incidence ratio (SIR) with their 95% confidence intervals (CIs), or sufficient data to calculate them; and f) they were of high quality according to the Newcastle–Ottawa scale (NOS) (more than 6)[35] or were assessed to have low risk of bias, using the recommended tool in the Cochrane Handbook for Systematic Reviews of Interventions.[36] Research on mortality rates of bladder cancer was not included because it could be unpredictably confounded by survival-related factors. We also did not consider studies in which the population included healthy people because diabetes was also considered to increase the risk of bladder cancer. If multiple publications from the same study population were available, the most recent and detailed study was entitled to be included in this meta-analysis.

Data extraction
Two authors extracted data independently by using a predefined data collection form, with disagreements being resolved by consensus. For each study, the following characteristics were collected: first author's name, year of publication, the country in which the study was carried out, participant characteristics (age and sex), study design, study population, range for follow-up, number of events and non-event subjects exposed to pioglitazone and comparison, medication use in comparison group, estimate effects with their 95% CIs and covariates adjusted for in the analysis. From each of the studies, we optionally excerpted the relative risk estimate that was adjusted for the greatest number of potential confounders. In dose effect analysis, we defined low dose as ≤8268 mg or ≤10500 mg or ≤14000 mg; moderate dose as 10501–28000 mg or 14001–40000 mg; high dose as >28000 mg or >40000 mg.

Quality assessment of searched papers was also undertaken independently by two authors according to the NOS for

observational studies, and Cochrane Tool Review Manager 5.3 for RCTs. The disagreements between authors were resolved by discussion and consensus.

Statistical methods

Studies that reported various measures of RR were included in this meta-analysis: rate ratio, HR, and SIR. In practice, these three measures of effect yield similar estimates of RR because the absolute risk of bladder cancer in patients with diabetes is low.

Overall RR estimates with their corresponding 95% CIs were calculated with the DerSimonian and Laird[37] random effects models, which consider both within-study and between-study variation. Heterogeneity among studies was measured by Q test and quantified by I^2 with higher value indicating a greater degree of heterogeneity. Random effects model was chosen to analyze the data.[38] Publication bias was assessed using Begg's test[39] (rank correlation method) and Egger's test (linear regression method).[40] $P<0.05$ was considered to be representative of a significant statistical publication bias. Dose effect analysis of pioglitazone was conducted if data were sufficient. Sensitivity analyses were also performed to assess the stability of obtained results with a single study deleted each time to manifest the influence of

the individual dataset to the pooled RR. All of the statistical analyses were performed with STATA 14.0 (StataCorp LP, College Station, TX, USA), using two-sided P-values.

Results

Literature search

Figure 1 outlines our study selection process. Briefly, after removing duplicates, the search strategy generated 561 articles. Of these, the majority was excluded after the first screening based on abstracts or titles, mainly because they were reviews, basic research, case–control studies, or obviously not relevant to our analysis. After full-text review of 21 papers, 14 studies were excluded for the following reasons: low NOS quality score;[17,18,20,41,42] outcomes were not bladder cancer;[43,44] duplicate population;[16,22] used rosiglitazone as reference group;[45] used non-DM people as reference group;[46,47] without reference;[11] obviously abnormal data (details of exclusion criteria in Table S1).[23] We also identified five publications through checking reference lists of retrieved studies and reviews. Thus, a total of 12 cohort studies or nested case–control studies,[7–10,12–15,19,23–25] which met the inclusion criteria, were included in the meta-analysis. Two RCT studies[26,27] were also identified in this process and were systematically reviewed.
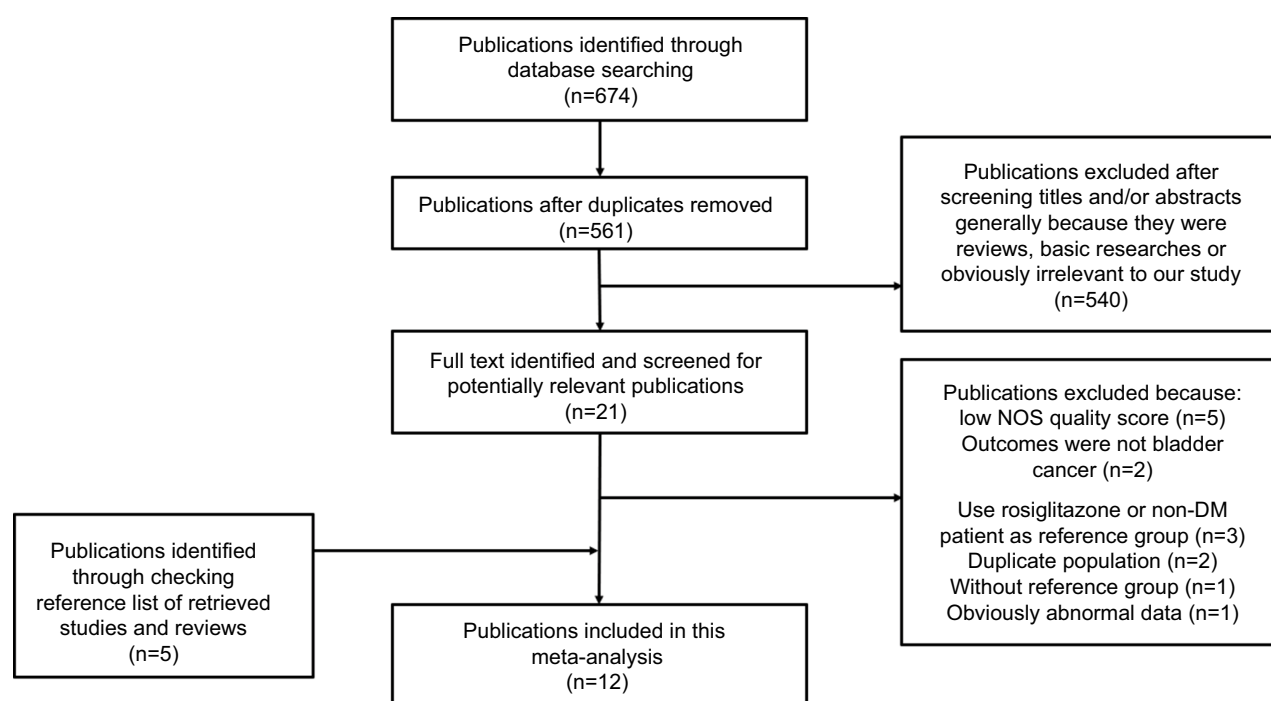

Figure 1 Flowchart of study assessment and selection.
Abbreviations: NOS, Newcastle–Ottawa scale; DM, diabetes mellitus.

Data obtained from RCTs

Two RCT studies, PROactive[26] and TOSCA.IT,[27] which reported bladder cancer cases in patients with diabetes, were eligible for this study. As it is meaningless to perform a meta-analysis for two studies, we systematically reviewed them. Characteristics including population, interventions, years of follow-up, and number of cases are shown in Table S2. Risk of bias assessment of the studies is demonstrated in Figure S1.

The PROactive study was performed in patients with T2DM who were aged 35–75 years. Among a total of 5238 patients who were enrolled in the PROactive study, 2605 patients were randomized to the pioglitazone group and 27 bladder cancer cases were identified finally after double-blind period +10-year observational follow-up. Meanwhile, 26 cases were reported in the placebo group which consisted of 2633 T2DM patients who took placebo. The authors found no significant association between pioglitazone and bladder cancer with RR and 95% CI 1.05 (0.61–1.79). The other study, TOSCA.IT, was a multicenter prospective, randomized, open label, blinded end point designed. The authors reported eight cases in metformin + pioglitazone (n=1535) and metformin + sulfonylurea group (n=1493). Similar to the PROactive study, no significant relationship was detected. Both studies were assessed to have a low risk of bias (Figure S1).

Data obtained from observational studies

Study characteristics

The characteristics of the 12 observational studies are presented in Table 1. Of these, eight[6,8,10,12,14,15,19,25] were cohort studies and four[7,9,13,24] were nested case–control studies. The studies were conducted in the following regions: Europe (n=5),[10,13–15,19] Asia (n=4),[6,7,9,24] and USA (n=3).[8,12,25] The study population in 12 studies consisted of both sexes. All included studies were published between 2012 and 2018. The cohort ranged in size from 878[24] to 1.01 million.[19] Medication use in comparison group was either "never used any TZD"[13,24] or "never used pioglitazone"[6–10,12,14,15,19] or "use of sulfonylureas".[25] Adjustments were made for potential confounders of more than two factors in all studies.

Pioglitazone use and risk of bladder cancer

The overall RR with its 95% CI showed a statistically slight but significant association between pioglitazone use and risk of bladder cancer (Figure 2, RR 1.14, 95% CI 1.03–1.26). There is no significant heterogeneity among these studies with Q=17.86, P=0.085, and I²=38.4%.

We also performed dose effect analysis of pioglitazone (Table 2). We defined low dose as ≤8268 mg or ≤10500 mg or ≤14000 mg; moderate dose as 10501–28000 mg or 14001–40000 mg; high dose as >28000 mg or >40000 mg. Although there were not statistically significant results, the risk of bladder cancer showed a rising trend when the dose of pioglitazone increased (low dose, RR 1.12, 95% CI 0.95–1.33; moderate dose, RR 1.20, 95% CI 0.99–1.46; high dose, RR 1.29, 95% CI 0.75–2.22).

Sensitivity analysis

Sensitivity analyses of the pooled RRs were performed to test the stability of the pooled results. All of the integrated RRs were once again calculated by means of the random effects model. When omitting each study in the meta-analysis, the pooled RRs (Table S3) always remained stable.

Publication bias

There was no evidence indicating significant publication bias either with the Begg's test (P=1.0) or with the Egger's test (Figure 3, P=0.90).

Discussion

The finding of this meta-analysis of 12 studies indicates that pioglitazone is associated with a 14% increased risk of bladder cancer (95% CI 1.04–1.26). Quantified Q test and I² test were carried out to assess the extent of heterogeneity among the included studies. No statistically significant heterogeneity was found among the included studies (I²=38.4%, P=0.085). Our conclusion was convincing because a fixed model showed a similar result (RR 1.05, 95% CI 1.01–1.10) and our heterogeneity was not significant. In sensitivity analysis we found that heterogeneity largely decreased (I²=0, P=0.591) when excluding Levin et al's[19] study. This result may be due to the difference in methodology of Levin et al's paper. Unlike other cohort studies, Levin used a multi-population pooled cohort to assess the risk of bladder cancer and use of pioglitazone in patients with type 2 diabetes. This might be the origin of the heterogeneity. Although the result of two RCT studies providing high-level evidence showed a null association between pioglitazone and bladder cancer, it is still not convincing because a total of only 35 bladder cancer cases were reported in the intervention group. During our search process, another high-quality RCT study assessing risk of bladder cancer and pioglitazone in patients without diabetes was identified.[47] Similar to two other RCTs, 12 bladder cancer cases in the pioglitazone group were reported and

Table 1 Characteristics of observational studies of pioglitazone and bladder cancer

Study	Age	Sex (percentage male)	Study design	Study population	Follow-up (years)	Medication use in comparison group	Exposed group Events	Exposed group No events	Control group Events	Control group No events	Covariates	NOS score
Azoulay et al,[13] 2012, UK	64.1	81.4	Nested case-control study	T2DM patients in GPRD between Jan 1, 1988, and Dec 31, 2009	4.6	Never used any of the TZDs	19	191	355	6452	Age, sex, entry date, follow-up, HbA1c, alcohol, obesity, smoking, bladder conditions, drugs	8
Neumann et al,[10] 2012, France	61.5	53.8 (exposed), 53.4 (control)	Cohort study	All DM patients aged 40–79 years in 2006 in SNIIRAM and PMSI	3.1	Never used pioglitazone	175	155360	1841	1333684	Age, sex, drugs	7
Hsiao et al,[9] 2012, Taiwan	68.5	68.5	Nested case-control study	T2DM patients in NHIRD between Jan 1, 1997, and Dec 31, 2008	3.6	Never used pioglitazone	153	523	3259	16537	Age, sex, entry date, duration of diabetes, comorbid conditions, medications	7
Vallarino et al,[12] 2013, USA	58.1	59.6 (exposed), 53.0 (control)	Cohort study	T2DM patients over 45 years in i3 InVision Data MartTM between May 1, 2000, and Jun 30, 2010	2.2 (exposed), 1.9 (control)	Never used pioglitazone	84	38475	44	17904	Age, sex, tobacco use, medications, medical history	8
Wei et al,[14] 2013, UK	62.9 (exposed), 64.8 (control)	57.0 (exposed), 54.1 (control)	Cohort study	T2DM patients over 40 years in GPRD between 2001 and 2010	3.5 (exposed), 5.3 (control)	Never used pioglitazone	66	23481	803	183363	Age, sex, duration of diabetes, smoking, BMI, drugs	7
Kuo et al,[7] 2014, Taiwan	69.6	61.8	Nested case-control study	T2DM patients in NHI from 1997 to 2009	NA	Never used pioglitazone	15	52	244	984	Age, sex, documented risk factors for bladder cancer, comorbidities	8
Lewis, et al,[8] 2015, USA	over 40	53.5	Cohort study	Diabetes patients over 40 years between 1997 and 2002 in KPNC	6.1 (exposed), 7.2 (control)	Never used pioglitazone	186	33995	1075	157843	Age, sex, ethnicity, smoking, creatinine, income, medications, bladder conditions, PSA, HBA1c, complications, comorbidities	9
Jin et al,[6] 2014, Korea	62.9 (exposed), 63.4 (control)	52.3 (exposed), 53.4 (control)	Cohort study	T2DM patients between Nov 2005 and Jun 2011 from four hospitals in Korea	NA	Never used pioglitazone	30	11210	237	101716	Age, sex	7
Korhonen et al,[15] 2016, Europe	63.2 (exposed), 66.6 (control)	56.3 (exposed), 53.7 (control)	Cohort study	All T2DM patients over 40 years in database from four European countries	9.2	Never used pioglitazone	130	56207	970	316139	Age, sex, medications, comorbidities	8
Levin et al,[19] 2015, Europe	60–64	52.5	Cohort study	T2DM patients in six populations since the introduction of TZDs in that center	4–7.4	Never used pioglitazone	117	NA	3131	NA	Age, calendar year, diabetes duration, smoking, cumulative exposure to pioglitazone	8
Han et al,[24] 2016, Korea	Over 40	81.2	Nested case-control study	T2DM patients who were first prescribed oral antidiabetic agents from 2003 to 2013 in NHIS-NSC	7.9	Never used any of the TZDs	5	52	69	752	Medications, medical history, Charlson comorbidity score, household income level, area	8
Garry et al,[25] 2018, USA	75	41	Cohort study	T2DM patients aged over 65 years between 2007 and 2014 in Medicare beneficiaries	7	Sulfonylureas	73	20002	387	125717	age, sex, and race	8

Abbreviations: NA, data not available; T2DM, type 2 diabetes mellitus; TZDs, thiazolidinedione; BMI, body mass index; NOS, Newcastle–Ottawa scale; GPRD, general practice research database; SNIIRAM, Système National d'Information Inter-régimes de l'Assurance Maladie (French national health insurance information system); PMSI, Programme de Médicalisation des Systèmes d'Information (French hospital discharge database); NHIRD, Taiwan's National Health Insurance Research Database; NHI, National Health Insurance; KPNC, Kaiser Permanente Northern California; NHIS-HSC, National Health Insurance Service National Sample Cohort; PSA, Prostate specific antigen.
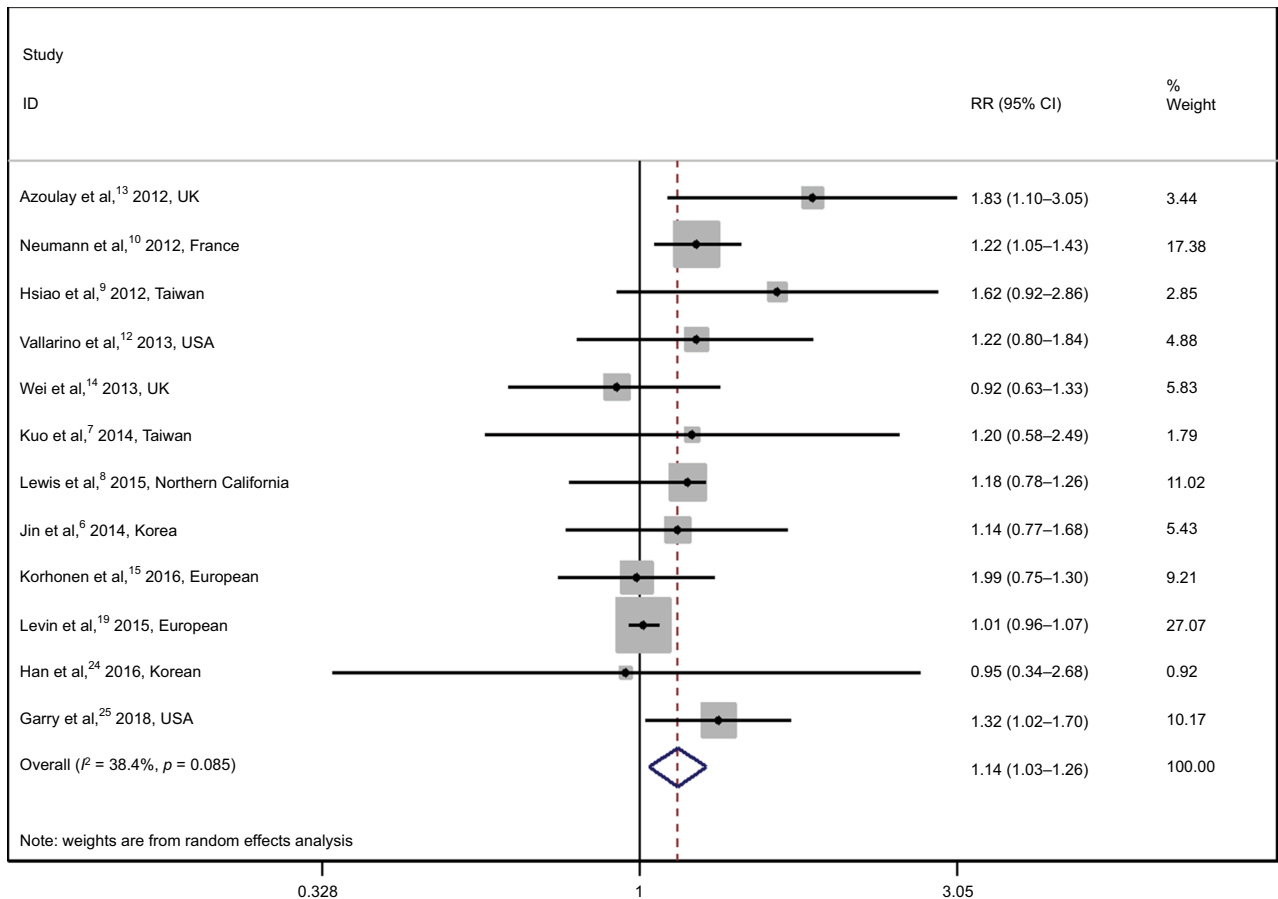
Figure 2 Relative risks for the association between pioglitazone use and risk of bladder cancer.
Notes: Diamonds represent study-specific relative risks (RRs) or summary relative risks with 95% confidence intervals (CIs). Horizontal lines represent 95% CIs. Test for heterogeneity among studies: P=0.085, I^2=38.4%.

Table 2 Dose effect analysis of relative risks for the association between pioglitazone and bladder cancer

Dose used	RR (95% CI)	Heterogeneity test		
		Q	**P**	**I^2 (%)**
Low	1.12 (0.95–1.33)	1	0.91	0
Moderate	1.20 (0.99–1.46)	2.5	0.47	0
High	1.29 (0.75–2.22)	9.4	0.02	67.9

Notes: Low dose is defined ≤8268 mg or ≤10500 mg or ≤14000 mg; moderate dose is defined 10501–28000 mg or 14001–40000 mg; high dose is defined >28000 mg or >40000 mg.
Abbreviations: RR, relative risk; CI, confidence interval.

no significant relationship was detected. In spite of its high quality, this study was excluded from the systematic review because the non-diabetic population did not fit our study goal.

One important molecular signaling pathway involved in carcinogenesis concerns of pioglitazone intake and risk of bladder cancer. Pioglitazone is a member of TZDs, which are agonists of PPARγ.[48] The effects of PPARγ agonists on human bladder cell lines have continually demonstrated significantly decreased proliferation, increased differentiation and apoptosis, suggesting that PPARγ is involved in antitumor action.[49–51] However, recent research reported by Yang et al indicates that higher expression of PPARγ or its activation by agonist TZD can promote bladder cancer cell migration and invasion,[52] suggesting that different expression levels of PPARγ may induce different outcomes. In light of this point, pioglitazone, the PPARγ agonist, might cause higher expression of PPARγ which promotes the process of carcinogenesis. In the future, more basic experimental research should be conducted to further elucidate the possible molecular mechanism of pioglitazone intake and risk of bladder cancer.

Our results of dose effect support this molecular theory. Patients who used a higher dose of pioglitazone had an increased risk of bladder cancer, indicating that larger amounts of pioglitazone may cause higher expression of PPARγ which promotes the process of carcinogenesis.

Additionally, no evidence of significant publication bias was observed in the analyses either with the Begg's test or
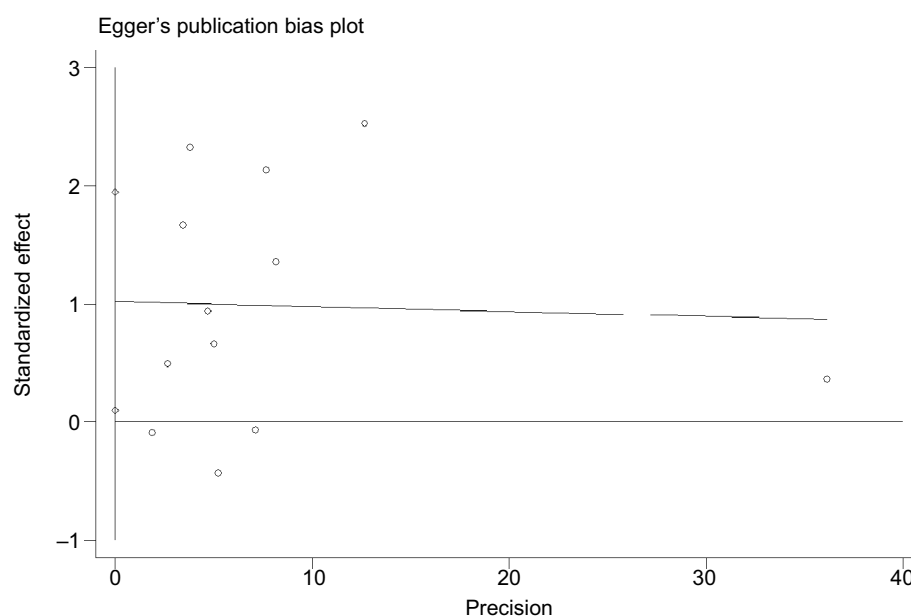
Egger's publication bias plot

Figure 3 Egger's publication bias plot.
Notes: Egger's regression asymmetry test (*P*=0.90). Standardized effect was defined as the odds ratio divided by its standard error. Precision was defined as the inverse of the standard error.

Egger's test. These results greatly improved the predictability and reliability of our meta-analysis.

Compared to other related meta-analyses, our meta-analysis evaluating the correlation between pioglitazone and risk of bladder cancer is more convincing because: a) a transparent and robust approach was taken to examine the evidence base, including adherence to PRISMA guidelines; b) we included the newest and the broadest studies, to the best of our knowledge, with rigid inclusion criteria restriction in which only RCTs and high-quality (NOS quality score >6) cohort studies or nested case–control studies were included. We did not include the other high-quality RCT by Kernan et al[47] because the study population excluded patients with type 2 diabetes, which did not fit our aim. One RCT had another quality assessment method[36] and was systematically reviewed rather than pooling with observational studies. A previous meta-analysis[33] simply pooled results of RCTs and observational studies which makes the conclusions unconvincing. c) Patients' outcomes were only recorded in reliable databases or using ICD codes. This approach ensured methodological rigor. d) We did not include case–control studies to avoid latent high selection and recall bias; e) previous meta-analyses[29,30] did not exclude studies with duplicate populations.[9,16,22]

Nevertheless, some limitations should be mentioned: a) we only pooled cohort studies and nested case–control studies without RCTs. Two RCTs,[26,27] which provided convincing

level 1 evidence, reported null association between pioglitazone use and bladder cancer, but there was only a total of 35 bladder cancer cases in the intervention group. More future RCT studies should be performed to provide more convincing evidence. b) Although no publication bias was found in our meta-analysis by either Egger's or Begg's test, the selection strategy of published studies and language limitation could bring about potential publication bias which may have affected our ultimate findings; c) the uncontrolled or unmeasured latent risk factors could have produced biases. We cannot rule out the possibility that remaining confounding factors could have affected the results. Recently, Lewis et al[54] reported that proteinuria testing could potentially cause unmeasured confounding in studies of pioglitazone and bladder cancer but most of the studies included in this meta-analysis[6,7,9,10,12–14] did not adjust for proteinuria testing. d) Although our heterogeneity was not significant, the results of our meta-analysis should be interpreted with caution because heterogeneity of our study was not unapparent (Q=17.86, *P*=0.085 and I²=38.4%).

In conclusion, this meta-analysis indicated that pioglitazone is associated with an increased risk of bladder cancer. Despite some study limitations, we still suggest that doctors should carefully assess the overall risks and benefits of pioglitazone. Further research should be conducted to confirm our findings and clarify the potential biological mechanisms.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

References

1. Antoni S, Ferlay J, Soerjomataram I, et al. Bladder cancer incidence and mortality: a global overview and recent trends. *Eur Urol.* 2017;71(1):96–108.
2. Murta-Nascimento C, Schmitz-Drager BJ, Zeegers MP, et al. Epidemiology of urinary bladder cancer: from tumor development to patient's death. *World J Urol.* 2007;25(3):285–295.
3. Xu X, Wu J, Mao Y, et al. Diabetes mellitus and risk of bladder cancer: a meta-analysis of cohort studies. *PloS One.* 2013;8(3):e58079.
4. McConkey DJ, Choi W, Ochoa A, Dinney CP. Intrinsic subtypes and bladder cancer metastasis. *Asian J Urol.* 2016;3(4):260–267.
5. Suzuki S, Arnold LL, Pennington KL, et al. Effects of pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, on the urine and urothelium of the rat. *Toxicol Sci.* 2010;113(2):349–357.
6. Jin SM, Song SO, Jung CH, et al. Risk of bladder cancer among patients with diabetes treated with a 15 mg pioglitazone dose in Korea: a multi-center retrospective cohort study. *J Korean Med Sci.* 2014;29(2):238–242.
7. Kuo HW, Tiao MM, Ho SC, Yang CY. Pioglitazone use and the risk of bladder cancer. *Kaohsiung J Med Sci.* 2014;30(2):94–97.
8. Lewis JD, Habel LA, Quesenberry CP, et al. Pioglitazone use and risk of bladder cancer and other common cancers in persons with diabetes. *JAMA.* 2015;314(3):265–277.
9. Hsiao FY, Hsieh PH, Huang WF, Tsai YW, Gau CS. Risk of bladder cancer in diabetic patients treated with rosiglitazone or pioglitazone: a nested case-control study. *Drug Saf.* 2013;36(8):643–649.
10. Neumann A, Weill A, Ricordeau P, et al. Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. *Diabetologia.* 2012;55(7):1953–1962.
11. Mackenzie TA, Zaha R, Smith J, Karagas MR, Morden NE. Diabetes pharmacotherapies and bladder cancer: a medicare epidemiologic study. *Diabetes Ther.* 2016;7(1):61–73.
12. Vallarino C, Perez A, Fusco G, et al. Comparing pioglitazone to insulin with respect to cancer, cardiovascular and bone fracture endpoints, using propensity score weights. *Clin Drug Investig.* 2013;33(9):621–631.
13. Azoulay L, Yin H, Filion KB, et al. The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. *BMJ.* 2012;344:e3645.
14. Wei L, MacDonald TM, Mackenzie IS. Pioglitazone and bladder cancer: a propensity score matched cohort study. *Br J Clin Pharmacol.* 2013;75(1):254–259.
15. Korhonen P, Heintjes EM, Williams R, et al. Pioglitazone use and risk of bladder cancer in patients with type 2 diabetes: retrospective cohort study using datasets from four European countries. *BMJ.* 2016;354:i3903.
16. Lee MY, Hsiao PJ, Yang YH, Lin KD, Shin SJ. The association of pioglitazone and urinary tract disease in type 2 diabetic Taiwanese: bladder cancer and chronic kidney disease. *PloS One.* 2014;9(1):e85479.
17. Balaji V, Seshiah V, Ashtalakshmi G, Ramanan SG, Janarthinakani M. A retrospective study on finding correlation of pioglitazone and incidences of bladder cancer in the Indian population. *Indian J Endocrinol Metab.* 2014;18(3):425–427.
18. Gupta S, Gupta K, Ravi R, et al. Pioglitazone and the risk of bladder cancer: an Indian retrospective cohort study. *Indian J Endocrinol Metab.* 2015;19(5):639–643.
19. Levin D, Bell S, Sund R, et al. Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. *Diabetologia.* 2015;58(3):493–504.
20. Fujimoto K, Hamamoto Y, Honjo S, et al. Possible link of pioglitazone with bladder cancer in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract.* 2013;99(2):e21–23.
21. Song SO, Kim KJ, Lee BW, et al. The risk of bladder cancer in korean diabetic subjects treated with pioglitazone. *Diabetes Metab J.* 2012;36(5):371–378.
22. Tseng CH. Pioglitazone and bladder cancer: a population-based study of Taiwanese. *Diabetes Care.* 2012;35(2):278–280.
23. Tuccori M, Filion KB, Yin H, et al. Pioglitazone use and risk of bladder cancer: population based cohort study. *BMJ.* 2016;352:i1541.
24. Han E, Jang SY, Kim G, et al. Rosiglitazone use and the risk of bladder cancer in patients with type 2 diabetes. *Medicine (Baltimore).* 2016;95(6):e2786.
25. Garry EM, Buse JB, Lund JL, Pate V, Sturmer T. Comparative safety of pioglitazone versus clinically meaningful treatment alternatives concerning the risk of bladder cancer in older US adults with type 2 diabetes. *Diabetes Obes Metab.* 2018;20(1):129–140.
26. Erdmann E, Harding S, Lam H, Perez A. Ten-year observational follow-up of PROactive: a randomized cardiovascular outcomes trial evaluating pioglitazone in type 2 diabetes. *Diabetes Obes Metab.* 2016;18(3):266–273.
27. Vaccaro O, Masulli M, Nicolucci A, et al. Effects on the incidence of cardiovascular events of the addition of pioglitazone versus sulfonylureas in patients with type 2 diabetes inadequately controlled with metformin (TOSCA.IT): a randomised, multicentre trial. *Lancet Diabetes Endocrinol.* 2017;5(11):887–897.
28. fda.gov [homepage on the Internet]. FDA Drug Safety Communication: Updated FDA review concludes that use of type 2 diabetes medicine pioglitazone may be linked to an increased risk of bladder cancer. US Food and Drug Administration; 2016. Available from: https://www.fda.gov/Drugs/DrugSafety/ucm519616. Accessed May 2, 2018.
29. Davidson MB, Pan D. An updated meta-analysis of pioglitazone exposure and bladder cancer and comparison to the drug's effect on cardiovascular disease and non-alcoholic steatohepatitis. *Diabetes Res Clin Pract.* 2017;135:102–110.
30. Filipova E, Uzunova K, Kalinov K, Vekov T. Pioglitazone and the risk of bladder cancer: a meta-analysis. *Diabetes Ther.* 2017;8(4):705–726.
31. Pai SA, Kshirsagar NA. Pioglitazone utilization, efficacy & safety in Indian type 2 diabetic patients: a systematic review & comparison with European Medicines Agency Assessment Report. *Indian J Med Res.* 2016;144(5):671–680.
32. Turner RM, Kwok CS, Chen-Turner C, et al. Thiazolidinediones and associated risk of bladder cancer: a systematic review and meta-analysis. *Br J Clin Pharmacol.* 2014;78(2):258–273.
33. He S, Tang YH, Zhao G, et al. Pioglitazone prescription increases risk of bladder cancer in patients with type 2 diabetes: an updated meta-analysis. *Tumor Biol.* 2014;35(3):2095–2102.
34. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med.* 2009;151(4):264–269.
35. Ottawa Hospital Research Institute [homepage on the Internet]. Wells GA, Shea B, O'Connell DO, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available from:http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed May 2, 2018.
36. Higgins JP, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration; 2011. Available from: http://handbook.cochrane.org. Accessed May 2, 2018.
37. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7(3):177–188.
38. Zeng D, Lin DY. On random-effects meta-analysis. *Biometrika.* 2015;102(2):281–294.

39. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics.* 1994;50(4):1088–1101.

40. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ.* 1997;315(7109):629–634.

41. Hsu YH, Yang YT, Chen PN, et al. Association between pioglitazone and bladder cancer among patients with type II diabetes: a propensity score matched cohort study. *Value in Health.* 2014;17(3):A73.

42. Piccinni C, Motola D, Marchesini G, Poluzzi E. Assessing the association of pioglitazone use and bladder cancer through drug adverse event reporting. *Diabetes Care.* 2011;34(6):1369–1371.

43. Lin HC, Hsu YT, Kachingwe BH, et al. Dose effect of thiazolidinedione on cancer risk in type 2 diabetes mellitus patients: a six-year population-based cohort study. *J Clin Pharm Ther.* 2014;39(4):354–360.

44. Kao CH, Sun LM, Chen PC, et al. A population-based cohort study in Taiwan--use of insulin sensitizers can decrease cancer risk in diabetic patients? *Ann Oncol.* 2013;24(2):523–530.

45. Mamtani R, Haynes K, Bilker WB, et al. Association between longer therapy with thiazolidinediones and risk of bladder cancer: a cohort study. *J Natl Cancer Inst.* 2012;104(18):1411–1421.

46. Bazelier MT, de Vries F, Vestergaard P, Leufkens HG, De Bruin ML. Use of thiazolidinediones and risk of bladder cancer: disease or drugs? *Curr Drug Saf.* 2013;8(5):364–370.

47. Kernan WN, Viscoli CM, Furie KL, et al. Pioglitazone after ischemic stroke or transient ischemic attack. *N Engl J Med.* 2016;374(14): 1321–1331.

48. Ching J, Amiridis S, Stylli SS, et al. The peroxisome proliferator activated receptor gamma agonist pioglitazone increases functional expression of the glutamate transporter excitatory amino acid transporter 2 (EAAT2) in human glioblastoma cells. *Oncotarget.* 2015;6(25): 21301–21314.

49. Wang Y, Tan H, Xu D, et al. The combinatory effects of PPAR-gamma agonist and survivin inhibition on the cancer stem-like phenotype and cell proliferation in bladder cancer cells. *Int J Mol Med.* 2014;34(1): 262–268.

50. Langle Y, Lodillinsky C, Belgorosky D, Sandes EO, Eijan AM. Role of peroxisome proliferator activated receptor-gamma in bacillus Calmette-Guerin bladder cancer therapy. *J Urol.* 2012;188(6):2384–2390.

51. Varley CL, Southgate J. Effects of PPAR agonists on proliferation and differentiation in human urothelium. *Exp Toxicol Pathol.* 2008;60(6):435–441.

52. Yang DR, Lin SJ, Ding XF, et al. Higher expression of peroxisome proliferator-activated receptor gamma or its activation by agonist thiazolidinedione-rosiglitazone promotes bladder cancer cell migration and invasion. *Urology.* 2013;81(5):1109.e1–6.

53. Lewis JD, Habel L, Quesenberry C, et al. Proteinuria testing among patients with diabetes mellitus is associated with bladder cancer diagnosis: potential for unmeasured confounding in studies of pioglitazone and bladder cancer. *Pharmacoepidemiol Drug Saf.* 2014;23(6): 636–645.

Supplementary materials

Table S1 Detailed reasons of excluded studies

Study	Reason
Balaji et al,[12] 2014	Low NOS quality score
Gupta et al,[13] 2015	Low NOS quality score
Fujimoto et al,[15] 2013	Low NOS quality score
Hsu et al,[22] 2014	Low NOS quality score
Piccinini et al,[23] 2011	Low NOS quality score
Lin et al,[24] 2014	Outcomes were not bladder cancer
Kao et al,[25] 2013	Outcomes were not bladder cancer
Lee et al,[11] 2014	Bladder cancer case cohort (n=84) from 2005 to 2009 in NHI was overlapped by Hsiao (n=3412) in NHI from 1997.1.1 to 2008.12.31
Tseng et al,[16] 2012	Bladder cancer case cohort (n=165) from 2006 to 2009 in NHI was overlapped by Hsiao (n=3412) in NHI from 1997.1.1 to 2008.12.31
Mamtani et al,[26] 2012	Use rosiglitazone as referred group
Bazelier et al,[27] 2013	Use non-DM people as referred group
Kernan et al,[28] 2016	Use non-DM people as referred group
Mackenzie et al,[6] 2016	Without referred group
Tuccori et al,[17] 2016	Bladder cancer incident rate in exposed group is 5.8% which is abnormally higher than other cohort studies

Abbreviations: DM, diabetes mellitus; NHI, National Health Insurance; NOS, Newcastle–Ottawa scale.

Table S2 Characters of included RCT studies

Study	Age	Sex (percentage of male)	Study population	Follow-up (year)	Intervention	Intervention group		Control group	
						Events	No events	Events	No events
Erdmann et al,[20] 2016, USA	62	66	T2DM patients between May 2001 and April 2002 from primary-care practices and diabetic or cardiovascular specialist departments in hospitals	10.7	Pioglitazone versus placebo	27	2578	26	2607
Vaccaro et al,[21] 2017, Italy	62.3	59	T2DM patients of at least 2 years' duration and were on stable treatment with full-dose metformin	4.8	Metformin plus pioglitazone group vs metformin plus sulfonylureas group	8	1527	8	1485

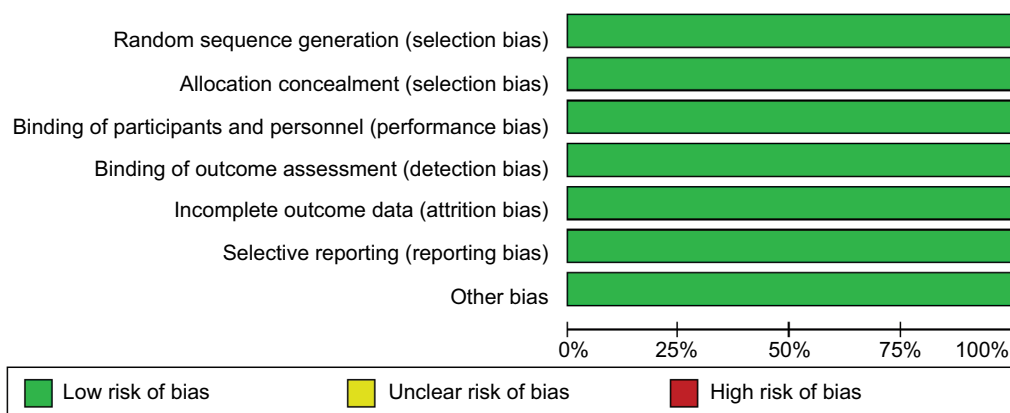**Abbreviations:** T2DM, type 2 diabetes mellitus; RCT, randomized controlled trial.

Figure S1 Risk of bias assessment of two RCT studies.
Abbreviation: RCT, randomized controlled trial.

Table S3 Sensitivity analysis of relative risks for the association between pioglitazone and bladder cancer

Study omitted	RR (95% CI)	Heterogeneity test		
		Q	P	I2 (%)
Azoulay et al,[8] 2012	1.11 (1.02–1.21)	13.33	0.206	25
Neumann et al,[5] 2012	1.12 (1.01–1.25)	14.09	0.169	29
Hsiao et al,[4] 2012	1.13 (1.02–1.24)	15.64	0.11	36.1
Wei et al,[9] 2013	1.14 (1.03–1.27)	17.38	0.066	42.5
Vallarino et al,[7] 2013	1.16 (1.04–1.29)	17.33	0.067	42.3
Kuo et al,[2] 2014	1.14 (1.03–1.27)	17.73	0.06	43.6
Lewis, et al,[3] 2015	1.14 (1.02–1.28)	16.98	0.075	41.1
Jin et al,[1] 2014	1.15 (1.03–1.28)	17.7	0.06	43.5
Korhonen et al,[10] 2016	1.16 (1.04–1.30)	17.65	0.061	43.3
Levin et al,[14] 2015	1.20 (1.09–1.31)	8.39	0.591	0
Han et al,[18] 2016	1.15 (1.03–1.27)	17.82	0.058	43.9
Garry et al,[19] 2018	1.12 (1.01–1.24)	14.78	0.14	32.4

References

1. Jin SM, Song SO, Jung CH, et al. Risk of bladder cancer among patients with diabetes treated with a 15 mg pioglitazone dose in Korea: a multi-center retrospective cohort study. *J Korean Med Sci*. 2014;29(2):238–242.
2. Kuo HW, Tiao MM, Ho SC, Yang CY. Pioglitazone use and the risk of bladder cancer. *Kaohsiung J Med Sci*. 2014;30(2):94–97.
3. Lewis JD, Habel LA, Quesenberry CP, et al. Pioglitazone use and risk of bladder cancer and other common cancers in persons with diabetes. *JAMA*. 2015;314(3):265–277.
4. Hsiao FY, Hsieh PH, Huang WF, Tsai YW, Gau CS. Risk of bladder cancer in diabetic patients treated with rosiglitazone or pioglitazone: a nested case-control study. *Drug Saf*. 2013;36(8):643–649.
5. Neumann A, Weill A, Ricordeau P, et al. Pioglitazone and risk of bladder cancer among diabetic patients in France: a population-based cohort study. *Diabetologia*. 2012;55(7):1953–1962.
6. Mackenzie TA, Zaha R, Smith J, Karagas MR, Morden NE. Diabetes pharmacotherapies and bladder cancer: a medicare epidemiologic study. *Diabetes Ther*. 2016;7(1):61–73.
7. Vallarino C, Perez A, Fusco G, et al. Comparing pioglitazone to insulin with respect to cancer, cardiovascular and bone fracture endpoints, using propensity score weights. *Clin Drug Investig*. 2013;33(9):621–631.
8. Azoulay L, Yin H, Filion KB, et al. The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. *BMJ*. 2012;344:e3645.
9. Wei L, MacDonald TM, Mackenzie IS. Pioglitazone and bladder cancer: a propensity score matched cohort study. *Br J Clin Pharmacol*. 2013;75(1):254–259.
10. Korhonen P, Heintjes EM, Williams R, et al. Pioglitazone use and risk of bladder cancer in patients with type 2 diabetes: retrospective cohort study using datasets from four European countries. *BMJ*. 2016;354:i3903.
11. Lee MY, Hsiao PJ, Yang YH, Lin KD, Shin SJ. The association of pioglitazone and urinary tract disease in type 2 diabetic Taiwanese: bladder cancer and chronic kidney disease. *PloS One*. 2014;9(1):e85479.
12. Balaji V, Seshiah V, Ashtalakshmi G, Ramanan SG, Janarthinakani M. A retrospective study on finding correlation of pioglitazone and incidences of bladder cancer in the Indian population. *Indian J Endocrinol Metab*. 2014;18(3):425–427.
13. Gupta S, Gupta K, Ravi R, et al. Pioglitazone and the risk of bladder cancer: an Indian retrospective cohort study. *Indian J Endocrinol Metab*. 2015;19(5):639–643.
14. Levin D, Bell S, Sund R, et al. Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. *Diabetologia*. 2015;58(3):493–504.

15. Fujimoto K, Hamamoto Y, Honjo S, et al. Possible link of pioglitazone with bladder cancer in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract*. 2013;99(2):e21–23.
16. Tseng CH. Pioglitazone and bladder cancer: a population-based study of Taiwanese. *Diabetes Care*. 2012;35(2):278–280.
17. Tuccori M, Filion KB, Yin H, et al. Pioglitazone use and risk of bladder cancer: population based cohort study. *BMJ*. 2016;352:i1541.
18. Han E, Jang SY, Kim G, et al. Rosiglitazone use and the risk of bladder cancer in patients with type 2 diabetes. *Medicine (Baltimore)*. 2016;95(6):e2786.
19. Garry EM, Buse JB, Lund JL, Pate V, Sturmer T. Comparative safety of pioglitazone versus clinically meaningful treatment alternatives concerning the risk of bladder cancer in older US adults with type 2 diabetes. *Diabetes Obes Metab*. 2018;20(1):129–140.
20. Erdmann E, Harding S, Lam H, Perez A. Ten-year observational followup of PROactive: a randomized cardiovascular outcomes trial evaluating pioglitazone in type 2 diabetes. *Diabetes Obes Metab*. 2016;18(3):266–273.
21. Vaccaro O, Masulli M, Nicolucci A, et al. Effects on the incidence of cardiovascular events of the addition of pioglitazone versus sulfonylureas in patients with type 2 diabetes inadequately controlled with metformin (TOSCA.IT): a randomised, multicentre trial. *Lancet Diabetes Endocrinol*. 2017;5(11):887–897.
22. Hsu YH, Yang YT, Chen PN, et al. Association between pioglitazone and bladder cancer among patients with type II diabetes: a propensity score matched cohort study. *Value in Health*. 2014;17(3):A73.
23. Piccinni C, Motola D, Marchesini G, Poluzzi E. Assessing the association of pioglitazone use and bladder cancer through drug adverse event reporting. *Diabetes Care*. 2011;34(6):1369–1371.
24. Lin HC, Hsu YT, Kachingwe BH, et al. Dose effect of thiazolidinedione on cancer risk in type 2 diabetes mellitus patients: a six-year populationbased cohort study. *J Clin Pharm Ther*. 2014;39(4):354–360.
25. Kao CH, Sun LM, Chen PC, et al. A population-based cohort study in Taiwan–use of insulin sensitizers can decrease cancer risk in diabetic patients? *Ann Oncol*. 2013;24(2):523–530.
26. Mamtani R, Haynes K, Bilker WB, et al. Association between longer therapy with thiazolidinediones and risk of bladder cancer: a cohort study. *J Natl Cancer Inst*. 2012;104(18):1411–1421.
27. Bazelier MT, de Vries F, Vestergaard P, Leufkens HG, De Bruin ML. Use of thiazolidinediones and risk of bladder cancer: disease or drugs? *Curr Drug Saf*. 2013;8(5):364–370.
28. Kernan WN, Viscoli CM, Furie KL, et al. Pioglitazone after ischemic stroke or transient ischemic attack. *N Engl J Med*. 2016;374(14):1321–1331.