Pioglitazone HCl (ACTOS)

Clinical Study No. 01-03-TL-OPI-524

Cohort Study of Pioglitazone and Bladder Cancer in Patients with Diabetes

Follow-up Report on Testing for Proteinuria with Data from January 1, 1997 to December 31, 2010

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Multiple studies have suggested an increased risk of bladder cancer among diabetic patients treated with pioglitazone, particularly for more than 2 years[1-3]. In an 8-year interim analysis of our study conducted at the Kaiser Permanente Northern California (KPNC) performed at the request of the United States Food and Drug Administration[4], we tested for confounding by several time updating variables, such as complications of diabetes and medications used to treat conditions associated with diabetes, that were not included in the 5-year interim analysis (Tables 1 and 2). In general, these variables were not confounders of the association between pioglitazone and bladder cancer. In many cases, the variables were associated with the incidence of bladder cancer, but nonetheless were not confounders as they were not associated with pioglitazone exposure. However, adjustment for the presence of a positive test for proteinuria resulted in the largest decrease in the strength of the association between use of pioglitazone and bladder cancer risk. For example, the hazard ratio for more than 4 years of treatment with pioglitazone without adjustment for proteinuria was 1.38 (95% CI 0.97-1.96), and after adjusting for proteinuria testing was 1.27 (95% CI 0.90-1.81). As with the other time updating variables, the magnitude of this confounding effect was small, and did not meet our *a priori* definition of a 10% change to be included in the final model. However, it raised the question as to whether detection bias could have contributed to the observed association between pioglitazone and bladder cancer in prior studies.

When the KPNC bladder cancer study was initiated, there was little knowledge in the medical community of a potential association between pioglitazone and bladder cancer. Therefore, an increased suspicion of cancer among pioglitazone treated patients early during the study period was unlikely. However, detection bias could have occurred if there was an unplanned increased testing for asymptomatic bladder cancer among patients treated with pioglitazone, such as increased urine testing (Figure 1).

Although hematuria is common among patients with bladder cancer, routine screening for bladder cancer in asymptomatic patients is not recommended as part of the care of patients with diabetes or the general population[5]. In contrast, among patients with diabetes mellitus, annual testing for proteinuria is recommended to allow for early identification of diabetic nephropathy[6]. Testing for proteinuria would not usually be thought of as a screening test for bladder cancer. However, if testing for proteinuria then triggers completion of a full urinalysis and potential detection of asymptomatic hematuria (as is the case in some practices[7]), the proteinuria test can be conceptualized as a first step for screening unintentionally for bladder cancer (Figure 1).

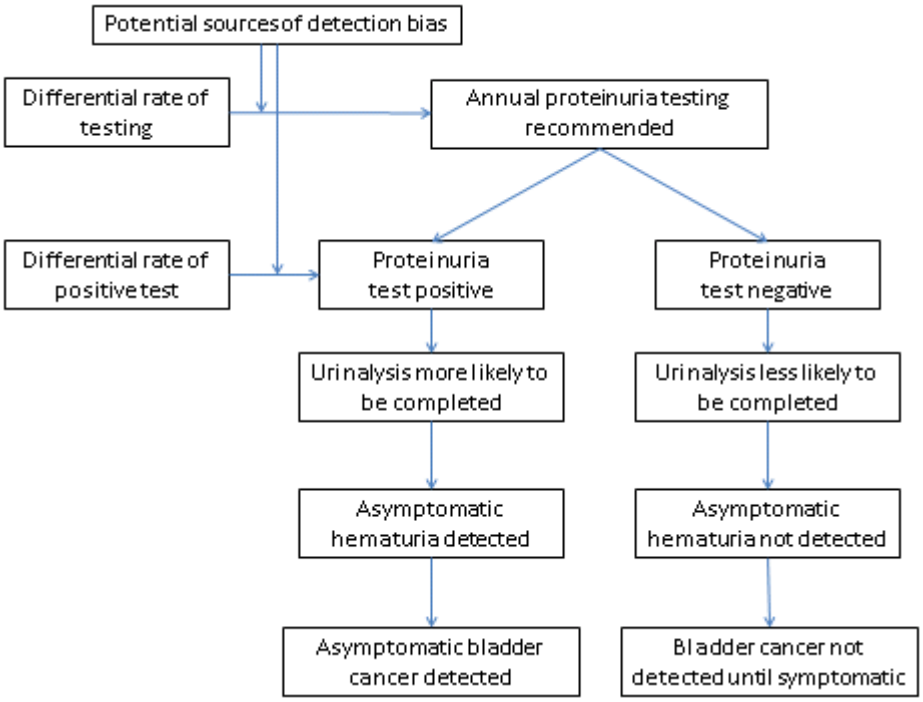

Figure 1. Conceptual model of how proteinuria testing could lead to detection bias. In this model, if patients treated with pioglitazone were more likely to be tested for proteinuria or if the test is more likely to be positive, this could lead to more frequent testing with urinalysis and a greater chance to detect asymptomatic bladder cancer.

In this report, we hypothesize that proteinuria testing may lead to detection bias in studies of the association between pioglitazone and bladder cancer if patients treated with pioglitazone are more likely to be tested for proteinuria and/or to have positive test results for proteinuria leading to a full urine analysis (Figure 1). To test this possibility, we investigated the following in the KPNC study of pioglitazone and bladder cancer: 1) whether testing for proteinuria is more common among diabetic patients treated with pioglitazone than among diabetic patients not treated with pioglitazone, 2) whether patients treated with pioglitazone are more likely to have a positive test for proteinuria, 3) whether a positive test for proteinuria is more likely to be associated with subsequent completion of a full urinalysis than a negative test for proteinuria, and 4) whether completion of a full urinalysis which includes hematuria testing is more likely to be associated with a subsequent diagnosis of bladder cancer than the lack of full urinalysis. If, the above diagnostic cascade is real, we hypothesized that detection bias could result from more frequent use of routine testing for proteinuria or a greater likelihood of having a positive test for proteinuria among patients treated with pioglitazone relative to other patients with diabetes. Therefore, as requested by the FDA, we have tested whether adjustment for testing for proteinuria, not just having a positive test, alters the previously observed association between use of pioglitazone and bladder cancer.

Methods

The study population has been previously described[3]. In brief, we included patients with diabetes who were included in the KPNC diabetes registry and who were 40 years or older as of

January 1, 1997 or reached age 40 prior to December 31, 2002. From this cohort of 207,389 we then excluded 823 patients with a diagnosis of bladder cancer prior to entry in the cohort or within 6 months of joining KPNC in order to avoid misclassification of prevalent bladder cancers as incident diagnoses. Likewise, patients without prescription benefits at the time of entry into the cohort (n=6,674) or those with a gap of more than four months in prescription or membership benefits where the gap started within the first four months of entering the cohort (n=6,782) were excluded. This resulted in 193,099 eligible men and women with diabetes mellitus. Exposure to pioglitazone and other diabetes medications was defined by the receipt of two prescriptions within a 6-month period. Patients were considered exposed from the date of the second prescription.

Diagnosis of bladder cancer was based on data included in the KPNC cancer registry, which is part of the Surveillance, Epidemiology and End Results (SEER) registry. We used the same definition of bladder cancer as previously reported[3].

Assessment of testing for proteinuria (both micro and macroalbuminuria) and complete urinalysis was determined from KPNC laboratory data files. Within KPNC clinical laboratories, when a spot microalbuminuria test is ordered, samples are initially tested for macroalbuminuria. Only if macroalbuminuria is not detected, then microalbuminuria testing is performed. A test was considered a full urinalysis (which includes testing for hematuria) if there was a full dipstick test or microscopic analysis completed.

In the first stage of our analysis, testing step #1 in the cascade, we examined the incidence of completing at least one test for proteinuria (with or without simultaneous testing for hematuria), stratified by whether the patient had been previously treated for diabetes with pioglitazone, only with medications other than pioglitazone, or with diet alone across each of the years 2000 to 2010. Within each calendar year, we separately determined whether testing was more common among patients treated with pioglitazone than compared to patients not treated with pioglitazone using logistic regression adjusting for patient age, sex, race, and smoking. We repeated this analysis examining the incidence of completing at least one full urinalysis, again stratified across the same groups.

For subsequent analyses, we needed to use only a single urine test for each subject. Because the rates of testing for proteinuria were relatively stable over time (see results), for efficiency, further analyses were conducted using only data from 2006 to 2008. In the second stage of the analysis, testing step #2 in the cascade, we compared the proportion of positive tests for proteinuria between patients who were and were not treated with pioglitazone using logistic regression, both unadjusted and adjusted for age, sex, race, and smoking status. Only the first test for proteinuria completed during 2006 to 2008 was used to maintain independence of all observations.

To determine whether a test for proteinuria that was positive was more likely to be followed by a full urinalysis than a negative test, step #3 in the cascade, we examined patients following their first test for proteinuria at any time from 2006 to 2008. In this analysis, the test for proteinuria could not include a test for hematuria on the same day (i.e. could not be a full urinalysis). From this cohort, we categorized the proteinuria test as positive (presence of micro or

macroalbuminuria) or negative. We then determined whether a full urinalysis was ordered more commonly during the 6 months after a proteinuria positive test than a negative test. We used logistic regression adjusted for age, sex, race, and smoking status to compute the adjusted odds ratio.

To assess whether a urinalysis was associated with a subsequent diagnosis with bladder cancer (step #4 in the cascade), we conducted an analysis among the patients with a positive test for proteinuria. Patients were categorized according to whether they had a urinalysis completed within 6 months of the first positive proteinuria test. The outcome variable was a diagnosis of bladder cancer within the 6 months following the test for proteinuria. If a diagnosis of bladder cancer was made within 6 months of the positive test for proteinuria but prior to completion of a urinalysis, the patient was considered not to have had a urinalysis. Logistic regression adjusted for age, sex, race, and smoking status was used to test the association between completion of a urinalysis and a subsequent diagnosis of bladder cancer among patients with a positive test for proteinuria.

Finally, we assessed the impact of adjusting for proteinuria testing on the association of pioglitazone with incident bladder cancer in two different Cox regression models treating proteinuria testing as a time updating variable. First, we categorized patients as exposed to testing for 1 year following completion of a test for proteinuria, after which they returned to the unexposed state unless an additional test was performed. In the second analysis, the variable was categorized as a 3-level variable: no testing, test completed and negative for proteinuria, test completed and positive for proteinuria. In this model, we again updated the variable at the time of each test for proteinuria and 1 year after the most recent testing. The Cox regression models were also adjusted for age, sex, calendar year of entry into the study, and only in the model of ever versus never exposure to pioglitazone for the use of other medications to treat diabetes.

Results

From the time of entry into the cohort to the end of follow-up, there were 2.00 million tests for proteinuria. These included random urine dipstick (49.2%), random urinary albumin to creatinine ratio (42.3%), random urinary protein to creatinine ratio (7.3%), 24 hours urine protein (1.1%, and 24 hour microalbuminuria testing (0.04%). Although the latter two tests, which entailed less than 1.2% of all tests, could be used as a confirmatory test or a primary test, we included these in our definition of testing to be comprehensive.

Step #1 of the cascade: Patients treated with pioglitazone were more likely to undergo testing for proteinuria (with or without simultaneous testing for hematuria) across all calendar years from 2000 to 2010 ($p<.001$ for all comparisons using logistic regression across all treatment groups) (Table 3). Patients who were not taking any medications for diabetes during the calendar year had the lowest rate of testing for proteinuria. For example, in 2010, testing was completed in 75%, 72%, and 59% of patients treated with pioglitazone, other medications, and no medications, respectively. To test whether the difference in testing was confounded, we

repeated the analysis for the year 2010 using logistic regression, adjusted for age, sex, race, and smoking. In this analysis, patients treated with pioglitazone were slightly more likely to have been tested for proteinuria than those treated with other drugs (adjusted OR=1.23, 95% CI 1.19-1.27) and much more likely to be tested than those treated with no diabetes drugs (age, sex, race, and smoking adjusted OR=2.48, 95% CI 2.36-2.60). Similar results were noted for completion of a full urinalysis and completion of a test for proteinuria that did not include testing for hematuria on the same day (Table 3).

Step #2 of the cascade: In the overall cohort of patients included in our 8-year interim report to the FDA, we observed a positive test for proteinuria in 74.3% of pioglitazone treated patients and 57.7% of patients not treated with pioglitazone ($p<0.01$). During the years 2006 to 2008, the proportion of tests for proteinuria that were positive was higher among the pioglitazone treated patients (unadjusted OR=1.36, 95 CI 1.32-1.41). There remained a significantly higher proportion of positive tests after adjusting for age, sex, race, and smoking status (OR= 1.41, 95 CI 1.36-1.46). Note that these tests may have included simultaneous testing for hematuria.

Step #3 of the cascade: Subsequently, we examined only those patients who had a test for proteinuria without testing for hematuria on the same day. In this cohort, a positive test for proteinuria was positively associated with completion of a urinalysis in the following 6 months (adjusted OR=1.78, 95% CI 1.73-1.85). Very similar results were observed in the patients treated with pioglitazone (adjusted OR 1.67, 95% CI 1.56-1.79) and those without exposure to pioglitazone (adjusted OR 1.78, 95% CI 1.72-1.85).

Step #4 of the cascade: Next, we confirmed that following a positive test for proteinuria, completion of a urinalysis is associated with a subsequent diagnosis of bladder cancer within the next 6 months following the positive test for proteinuria (adjusted OR=16.7, 95% CI 6.4 – 43.6).

Assessment for confounding by testing for proteinuria: When we included testing for proteinuria (with or without simultaneous testing for hematuria) in our Cox regression models as a dichotomous variable, testing was strongly associated with diagnosis of bladder cancer within the next 1 year (HR=17.6, 95% CI 12.6-24.8). Inclusion of testing as a 3-level variable demonstrated that the association was influenced by whether the test for proteinuria was positive or negative. The adjusted hazard ratio for a negative test result was (HR=11.8, 95% CI 8.32-16.6) while for a positive test result the hazard ratio was (HR=30.9, 95% CI 21.9-43.7). Table 4 demonstrates the effect of inclusion of this 3-level variable on the association of pioglitazone exposure and incident bladder cancer. In each of the analyses, inclusion of the 3-level variable shifted the hazard ratio for pioglitazone toward the null, reaching a 10% threshold of change only for analyses of time since initiating treatment with pioglitazone. The hazard ratio for more than 4 years of therapy with pioglitazone shifted from 1.38 in models adjusted only for age, sex, and calendar year of cohort entry to 1.26 after additional adjustment for proteinuria testing.

Because proteinuria could be detected when ordering a full urinalysis for other reasons, we repeated the analysis of the association between pioglitazone exposure and bladder cancer

adjusting only for testing for proteinuria when there was no test for microscopic hematuria performed on the same day. Of all tests for proteinuria, 49% had a test for microscopic hematuria on the same day. In this analysis, the adjusted hazard ratio for a negative proteinuria test result was 0.63 (95% CI 0.52-0.75) while for a positive proteinuria test result the hazard ratio was 2.45 (95% CI 2.12-2.82). Table 4 demonstrates the effect of inclusion of this 3-level variable on the association of pioglitazone exposure and incident bladder cancer. Again, the hazard ratios for pioglitazone moved closer to unity, but the magnitude of change was generally small.

Discussion

Our 5-year planned interim analysis of this cohort demonstrated an association between pioglitazone exposure and incident bladder cancer with evidence of increasing association with longer-term therapy. In another interim analysis completed after 8 years of the study, the associations were weaker and no longer statistically significant. However, the point estimates remained greater than unity and the largest hazard ratios were again observed in those with the longest duration of therapy. Several other observational studies have suggested an association between pioglitazone exposure and bladder cancer, some of which were recently included in a meta-analysis[1]. However, none of the prior studies has been able to assess for the impact of testing for proteinuria. In this new analysis, we demonstrate the potential confounding effect of testing for proteinuria on the association between pioglitazone and bladder cancer and that the association between testing for proteinuria and the risk of bladder cancer depends on the results of the proteinuria test. In each of our models, inclusion of proteinuria testing, particularly when the testing was further stratified according to whether or not proteinuria was detected, resulted in a reduction in the magnitude of the association between pioglitazone and bladder cancer.

Detection bias results when there is differential testing for cancer between study groups that leads to a lower probability of diagnosing asymptomatic cancer or delayed diagnosis of symptomatic cancer in one group relative to the comparator. Possible causes for detection bias include increased suspicion for cancer or increased use of screening tests in one group relative to the other. In our conceptual model, we hypothesized that detection bias could result if patients treated with pioglitazone were more frequently tested for proteinuria or were more likely to have a positive proteinuria test. We hypothesize that both of these are possible because pioglitazone is not typically used as a first line therapy for diabetes mellitus. Therefore, patients treated with pioglitazone could have longer standing or more difficult to control diabetes than patients treated with commonly used first line therapies. In turn, this may result in patients treated with pioglitazone being more likely to undergo testing for proteinuria or to have a positive test for micro or macroalbuminuria. To confirm this hypothesis, we completed a series of analyses that documented a potential pathway between pioglitazone exposure, proteinuria testing, and subsequent bladder cancer diagnosis (Table 5). In this study, we have demonstrated that 1) testing for proteinuria was slightly more common among patients treated with pioglitazone than among those treated with other diabetes medications and least common among patients receiving no medications for diabetes, 2) that the rate of testing for proteinuria was relatively stable during the years 2000 to 2010 within KPNC, 3) that among patients tested

for proteinuria, those receiving pioglitazone were more likely to have a positive test, 4) that patients were more likely to undergo a full urinalysis during the 6 months following a positive test for proteinuria than following a negative test, and 5) that completion of a full urinalysis was associated with a subsequent diagnosis of bladder cancer.

When deciding whether to adjust for a potential confounder, one must consider whether the variable is within the causal pathway between the exposure and the outcome. Adjusting for a variable in the causal pathway will mask a true association while adjusting for a confounder will eliminate bias from the nuisance variable. Some have hypothesized that renal insufficiency and albuminuria may directly contribute to the incidence of bladder cancer[8-11]. Although rare cases of nephrotic syndrome have been reported among non-diabetic patients taking rosiglitazone[12], pioglitazone would not be expected to cause proteinuria[13]. Rather, both pioglitazone and rosiglitazone appear to reduce proteinuria among patients with diabetes[13]. Despite this, among patients tested for proteinuria, those treated with pioglitazone were more likely to have a positive test for proteinuria, presumably because they had more advanced diabetes.

An alternative explanation is that symptoms of bladder cancer prompt detection of proteinuria, which occurs before the diagnosis of bladder cancer is confirmed[11]. In that case, adjusting for proteinuria in our model would potentially bias us toward the null, thereby potentially missing a true association between pioglitazone and bladder cancer. To specifically address this possibility, we analyzed the relationship between testing for proteinuria without simultaneous testing for hematuria and the subsequent diagnosis of bladder cancer. Tests that were done exclusively for proteinuria are likely to be screening tests for diabetic nephropathy. Here we again observed a positive association between a positive test for proteinuria and a subsequent diagnosis of bladder cancer. However, if the test for proteinuria was negative, the patients were less likely to be diagnosed with bladder cancer than even those patients without testing for proteinuria.

Jorgensen et al. observed that albuminuria was a marker of future bladder cancer risk[11]. Furthermore, albuminuria was associated with a future cancer diagnosis even up to 4 years prior to the cancer diagnosis[11]. Of course, the vast majority of proteinuria among diabetic patients is not due to bladder cancer. Among the general population, less than 1.5% of those with asymptomatic proteinuria were found to have a genitourinary malignancy[14-16]. Nonetheless, we observed that following a test for only proteinuria, those with a positive test were more likely to have a subsequent urinalysis. This implies the potential for bias if testing for proteinuria or if the likelihood of a positive test for proteinuria differs between study groups, both of which were evident here. Thus, it appears that differential testing for proteinuria and differential likelihood of having a positive test could partially explain the previously observed associations between pioglitazone and bladder cancer.

Weiss has recommended that one should adjust for a cancer screening test if either the screening test has the ability to identify not only the cancer but treatable antecedents of the cancer or if the number of cancers included in the study would have been smaller if there was no screening[17]. The first scenario does not apply to bladder cancer. The second scenario might apply to studies of pioglitazone and bladder cancer since some bladder cancers may have an

asymptomatic phase such that these patients would have undiagnosed bladder cancer were it not for detection triggered by urine testing. In such a case, it has been recommended to adjust for use of the screening test during the time period when a cancer would be detectable but asymptomatic[17]. This entails use of a time updating variable approach as we employed here. We used a one year time period for patients to be considered tested for proteinuria, although the true duration of the detectable but asymptomatic period for bladder cancer is uncertain. Furthermore, Weiss has emphasized the importance of distinguishing tests completed to evaluate symptoms of cancer (i.e., diagnostic tests) from those performed as a screening test and only adjusting for screening tests. Our results showing massively elevated hazard ratios for even a negative proteinuria test when including urinalysis in the battery of tests for proteinuria are consistent with this. Thus, these results suggest that for studies of bladder cancer among patients with diabetes, it is more appropriate to adjust for proteinuria testing in the absence of a simultaneous test for hematuria than to adjust for any testing for proteinuria including a urinalysis with simultaneous testing for hematuria.

It is noteworthy that annual testing for microalbuminuria is recommended for all patients with diabetes mellitus. Within KPNC the rates of testing are quite high. Although testing was least common among patients who were not taking medications for their diabetes, the rates of testing among patients treated with pioglitazone were only 3% - 5% higher than in patients treated with medications other than pioglitazone. In the KPNC cohort, the patients treated with pioglitazone had a similar duration of diabetes as the other patients but were more likely to have documented diabetic complications (data shown in our prior report). This may reflect more advanced diabetes (which would explain the higher proportion of positive tests for proteinuria[18]) or more extensive testing for complications as with proteinuria.

We assessed whether pioglitazone use was associated with a positive test among those who underwent proteinuria testing at least once between from 2006 to 2008. During this time period, we selected only the first test completed for each patient, which allowed us to maintain full independence of each observation. The choice of years 2006 to 2008 was arbitrary, but it is unlikely to have biased the results for several reasons. First, the rate of testing in the cohort was relatively stable across time. Second, patients began use of pioglitazone at various time points during the study. Therefore, even within the period from 2006 to 2008, there were some patients who had been treated with pioglitazone for long periods of time and others who had only short term exposure.

The availability of complete laboratory data within KPNC is a unique resource that is available in very few data sources. As such, analyses such as the ones completed here cannot be easily reproduced in other studies. Furthermore, while there are readily available methods to assess the impact of an unmeasured confounder in sensitivity analyses, these methods are generally designed for time invariant dichotomous variables rather than time updating 3-level variables as we used here. Claims data may allow for adjustment for testing for proteinuria but not for the 3-level variable included in our models. Inclusion of the variable for testing will provide some level of adjustment and should be considered in studies where this variable is available.

It is important for investigators to look for signs of possible detection bias. Although our work here suggests that proteinuria testing could be a source of detection bias, the magnitude of confounding when we adjusted for proteinuria testing is small and other analyses that we have performed are less consistent with detection bias. An increased risk shortly after the onset of therapy and before such an increase is biologically plausible can be a signal that detection bias may be present. In our KPNC study, we have generally seen an increasing risk with longer pioglitazone therapy. Another signal of possible detection bias is an excess of early stage cancers. The KPNC study is unique in the availability of data on stage at diagnosis. In our 8-year interim report, we observed that the strength of association between pioglitazone and bladder cancer was greater when we excluded in situ cancers and papillary neoplasms of uncertain malignant potential from the outcome definition, which argues against detection bias. Thus, while our conceptual model supports a possible role for detection bias related to proteinuria testing, the totality of the evidence suggests that if any such bias is present in the KPNC study, it is likely of a small magnitude. However, the magnitude of confounding by a variable is unique to each study population, so while the effect was small in this cohort, it could be larger in others.

In summary, nearly all prior studies addressing the association of pioglitazone exposure and incident bladder cancer have lacked data on testing for proteinuria. The results of this analysis suggest that this may be an unmeasured confounder and that failure to adjust for this confounder may bias the results away from the null and toward a positive association between pioglitazone exposure and bladder cancer, although the magnitude of such bias is likely small in our study. In healthcare settings where urinalysis including testing for hematuria is utilized as a screening test for proteinuria, such adjustment may not be feasible unless there is a reliable way to distinguish tests done for screening from those done to evaluate symptoms. Furthermore, because the direction of the association of proteinuria testing with bladder cancer incidence depends on proteinuria test results, appropriate adjustment for proteinuria testing likely requires knowledge of the test results. As such, we recommend that when possible, investigators assess for possible confounding by proteinuria testing, treating this as a time updating variable that accounts both for whether the test was completed and the results of the test. We will use this method in our final analysis of the KPNC cohort.

Table 1. Relative Hazard (95% CI) of Bladder Cancer for Pioglitazone and Potential Time Updating Confounders

	Unadjusted	**Based model adjusted for age, sex and year of cohort entry**	**Base model + statin use**	**Base model + ACE-I or ARB use**	**Base model + medications for BPH**	**Base model + PSA testing**
Unexposed to pioglitazone	Reference	Reference	Reference	Reference	Reference	Reference
Ever exposed to pioglitazone	0.98 (0.81-1.18)	1.06 (0.87 - 1.30)*	1.06 (0.87 - 1.29)	1.06 (0.87-1.29)	1.06 (0.87-1.29)	1.06 (0.87-1.29)
New variable	N/A	N/A	1.11 (0.96-1.28)	1.20 (1.04-1.40)	1.59 (1.37-1.84)	2.01 (1.66-2.44)
Time since starting pioglitazone						
Less than 3.5 years	0.84 (0.65-1.08)	0.98 (0.76-1.26)	0.97 (0.75-1.24)	0.96 (0.75-1.24)	0.97 (0.76-1.25)	0.97 (0.75-1.24)
3.5-6.5 years	1.00 (0.73-1.37)	1.12 (0.82-1.54)	1.11 (0.81-1.52)	1.11 (0.81-1.52)	1.11 (0.81-1.52)	1.12 (0.82-1.53)
More than 6.5 years	1.21 (0.81-1.80)	1.29 (0.87-1.93)	1.29 (0.86-1.92)	1.29 (0.86-1.92)	1.28 (0.86-1.91)	1.30 (0.87-1.94)
New variable	N/A	N/A	1.12 (0.97-1.29)	1.21 (1.04-1.39)	1.59 (1.37-1.85)	2.01 (1.66-2.43)
Duration of therapy						
Less than 1.5 years	0.67 (0.50-0.91)	0.80 (0.60-1.08)	0.80 (0.59-1.08)	0.80 (0.59-1.07)	0.80 (0.60-1.08)	0.80 (0.59-1.07)
1.5-4.0 years	1.07 (0.81-1.41)	1.19 (0.91-1.57)	1.19 (0.90-1.57)	1.19 (0.90-1.56)	1.20 (0.91-1.58)	1.18 (0.90-1.56)
More than 4 years	1.28 (0.90-1.81)	1.38 (0.97-1.96)	1.38 (0.97-1.96)	1.38 (0.97-1.96)	1.37 (0.97-1.95)	1.37 (0.97-1.95)
New variable	N/A	N/A	1.12 (0.97-1.29)	1.21 (1.04-1.40)	1.59 (1.37-1.85)	2.01 (1.66-2.43)

*Also adjusted for use of other diabetes medication.

Table 1 (continued)

	Unadjusted	Base model adjusted for age, sex and year of cohort entry	Base model + statin use	Base model + ACE-I or ARB use	Base model + medications for BPH	Base model + PSA testing
Cumulative dose						
1 – 13000 mg	0.77 (0.58-1.03)	0.91 (0.69-1.22)	0.91 (0.69-1.22)	0.91 (0.68-1.21)	0.92 (0.69-1.22)	0.91 (0.68-1.21)
13001 – 35000 mg	0.91 (0.67-1.24)	1.01 (0.74-1.38)	1.01 (0.74-1.37)	1.00 (0.73-1.37)	1.01 (0.74-1.38)	1.00 (0.73-1.36)
>35000 mg	1.21 (0.88-1.65)	1.34 (0.98-1.83)	1.34 (0.98-1.83)	1.34 (0.98-1.83)	1.34 (0.98-1.83)	1.33 (0.97-1.82)
New variable	N/A	N/A	1.12 (0.97-1.29)	1.21 (1.04-1.39)	1.59 (1.37-1.85)	2.01 (1.65-2.43)

*Also adjusted for use of other diabetes medication.

Table 1 (continued)

	Unadjusted	Based model adjusted for age, sex and year of cohort entry	Base model + complications of diabetes	Base model + retinopathy	Base model + peripheral vascular disease	Base model + coronary artery disease
Unexposed to pioglitazone	Reference	Reference	Reference	Reference	Reference	Reference
Ever exposed to pioglitazone	0.98 (0.81-1.18)	1.06 (0.87 - 1.30)*	1.07 (0.87-1.30)	1.07 (0.88-1.31)	1.06 (0.87-1.30)	1.06 (0.87-1.29)
New variable	N/A	N/A	4.28 (3.29-5.57)	0.83 (0.71-0.96)	1.10 (0.96-1.25)	1.33 (1.17-1.51)
Time since starting pioglitazone						
Less than 3.5 years	0.84 (0.65-1.08)	0.98 (0.76-1.26)	0.93 (0.73-1.20)	0.99 (0.77-1.27)	0.98 (0.76-1.26)	0.97 (0.76-1.25)
3.5-6.5 years	1.00 (0.73-1.37)	1.12 (0.82-1.54)	1.10 (0.81-1.51)	1.15 (0.84-1.58)	1.12 (0.82-1.54)	1.11 (0.81-1.52)
More than 6.5 years	1.21 (0.81-1.80)	1.29 (0.87-1.93)	1.31 (0.87-1.95)	1.34 (0.89-2.00)	1.29 (0.87-1.93)	1.27 (0.85-1.89)
New variable	N/A	N/A	4.21 (3.25-5.47)	0.86 (0.74-0.99)	1.10 (0.96-1.26)	1.34 (1.18-1.52)
Duration of therapy						
Less than 1.5 years	0.67 (0.50-0.91)	0.80 (0.60-1.08)	0.77 (0.57-1.04)	0.82 (0.60-1.10)	0.80 (0.59-1.08)	0.79 (0.59-1.07)
1.5-4.0 years	1.07 (0.81-1.41)	1.19 (0.91-1.57)	1.16 (0.88-1.53)	1.22 (0.92-1.60)	1.19 (0.91-1.57)	1.18 (0.90-1.56)
More than 4 years	1.28 (0.90-1.81)	1.38 (0.97-1.96)	1.38 (0.97-1.96)	1.41 (0.99-2.00)	1.38 (0.97-1.95)	1.37 (0.97-1.95)
New variable	N/A	N/A	4.22 (3.25-5.48)	0.86 (0.74-0.99)	1.10 (0.97-1.26)	1.34 (1.18-1.52)

*Also adjusted for use of other diabetes medication.

Table 1 (continued)

	Unadjusted	Base model adjusted for age, sex and year of cohort entry	Base model + complications of diabetes	Base model + retinopathy	Base model + peripheral vascular disease	Base model + coronary artery disease
Cumulative dose						
1 – 13000 mg	0.77 (0.58-1.03)	0.91 (0.69-1.22)	0.88 (0.66-1.17)	0.93 (0.70-1.24)	0.91 (0.68-1.22)	0.90 (0.68-1.21)
13001 – 35000 mg	0.91 (0.67-1.24)	1.01 (0.74-1.38)	0.98 (0.72-1.34)	1.03 (0.75-1.40)	1.01 (0.74-1.37)	1.00 (0.73-1.36)
>35000 mg	1.21 (0.88-1.65)	1.34 (0.98-1.83)	1.33 (0.97-1.81)	1.37 (1.00-1.88)	1.34 (0.98-1.83)	1.33 (0.97-1.82)
New variable	N/A	N/A	4.21 (3.24-5.47)	0.86 (0.74-0.99)	1.10 (0.96-1.26)	1.34 (1.18-1.52)

*Also adjusted for use of other diabetes medication.

Table 1 (continued)

	Unadjusted	Based model adjusted for age, sex and year of cohort entry	Base model + HbA1c	Base model + proteinuria†
Unexposed to pioglitazone	Reference	Reference	Reference	Reference
Ever exposed to pioglitazone	0.98 (0.81-1.18)	1.06 (0.87 - 1.30)*	1.06 (0.87-1.29)	1.01 (0.83-1.23)
New variable	N/A	N/A	See table 2	5.71 (4.83-6.75)
Time since starting pioglitazone				
Less than 3.5 years	0.84 (0.65-1.08)	0.98 (0.76-1.26)	0.99 (0.77-1.27)	0.87 (0.67-1.12)
3.5-6.5 years	1.00 (0.73-1.37)	1.12 (0.82-1.54)	1.13 (0.83-1.55)	1.00 (0.73-1.37)
More than 6.5 years	1.21 (0.81-1.80)	1.29 (0.87-1.93)	1.30 (0.87-1.95)	1.17 (0.79-1.75)
New variable	N/A	N/A	See table 2	5.48 (4.64-6.46)
Duration of therapy				
Less than 1.5 years	0.67 (0.50-0.91)	0.80 (0.60-1.08)	0.83 (0.61-1.11)	0.71 (0.52-0.95)
1.5-4.0 years	1.07 (0.81-1.41)	1.19 (0.91-1.57)	1.20 (0.91-1.58)	1.06 (0.81-1.40)
More than 4 years	1.28 (0.90-1.81)	1.38 (0.97-1.96)	1.36 (0.96-1.93)	1.27 (0.90-1.81)
New variable	N/A	N/A	See table 2	5.49 (4.65-6.47)

*Also adjusted for use of other diabetes medication.

† Proteinuria is a subset of the variable for complications of diabetes

Table 1 (continued)

	Unadjusted	Base model adjusted for age, sex and year of cohort entry	Base model + HbA1c	Base model + proteinuria†
Cumulative dose				
1 – 13000 mg	0.77 (0.58-1.03)	0.91 (0.69-1.22)	0.94 (0.70-1.25)	0.81 (0.61-1.08)
13001 – 35000 mg	0.91 (0.67-1.24)	1.01 (0.74-1.38)	1.02 (0.75-1.39)	0.90 (0.66-1.22)
>35000 mg	1.21 (0.88-1.65)	1.34 (0.98-1.83)	1.34 (0.98-1.84)	1.22 (0.89-1.67)
New variable	N/A	N/A	See table 2	5.48 (4.65-6.47)

*Also adjusted for use of other diabetes medication.

† Proteinuria is a subset of the variable for complications of diabetes

Table 2. Relative Hazard (95% CI) of bladder cancer for HbA1c concentration when included in the model as a time updating confounders in the base model

	Ever exposed to pioglitazone	Time since starting pioglitazone	Duration of Therapy	Cumulative Dose
HbA1C <7	reference	reference	reference	reference
HbA1C 7-7.9	0.89 (0.76-1.03)	0.90 (0.78-1.04)	0.90 (0.78-1.04)	0.90 (0.78-1.04)
HbA1C 8-8.9	0.86 (0.70-1.05)	0.88 (0.72-1.07)	0.88 (0.72-1.08)	0.88 (0.72-1.08)
HbA1C 9-9.9	0.72 (0.53-0.98)	0.73 (0.54-1.00)	0.74 (0.55-1.01)	0.74 (0.54-1.00)
HbA1C >=10	0.52 (0.36-0.76)	0.53 (0.36-0.77)	0.53 (0.37-0.78)	0.53 (0.37-0.77)
Missing	0.56 (0.40-0.79)	0.56 (0.40-0.79)	0.57 (0.41-0.79)	0.57 (0.40-0.79)

Table 3. Incidence of completing at least one test for proteinuria

	Adjusted incidence of proteinuria testing without same day testing for hematuria[#] (95% CI)*			**Adjusted incidence of proteinuria testing with same day testing for hematuria† (95% CI)***			**Adjusted incidence of proteinuria testing with or without same day testing for hematuria[‡] (95% CI)***		
YEAR	**Pioglitazone**	**Other medications**	**No medications**	**Pioglitazone**	**Other medications**	**No medications**	**Pioglitazone**	**Other medications**	**No medications**
2000	60.1 (58.6-61.6)	51.0 (50.7-51.4)	37.7 (37.0-38.4)	40.8 (39.3-42.3)	33.4 (33.0-33.7)	28.4 (27.7-29.0)	73.7 (72.3-75.0)	65.3 (65.0-65.7)	53.3 (52.6-54.0)
2001	64.5 (63.4-65.5)	58.7 (58.3-59.0)	43.8 (43.1-44.5)	36.3 (35.2-37.4)	31.9 (31.6-32.2)	27.3 (26.7-27.9)	76.1 (75.1-77.0)	70.6 (70.3-70.9)	57.6 (56.9-58.3)
2002	67.5 (66.6-68.4)	62.9 (62.6-63.2)	51.2 (50.6-51.8)	35.8 (34.9-36.7)	31.2 (30.8-31.5)	27.0 (26.4-27.5)	78.0 (77.3-78.8)	73.9 (73.6-74.2)	63.5 (62.9-64.1)
2003	69.6 (68.8-70.3)	65.8 (65.5-66.1)	54.5 (53.9-55.1)	35.4 (34.6-36.2)	29.9 (29.6-30.2)	26.5 (26.0-27.1)	79.8 (79.1-80.5)	75.5 (75.2-75.8)	65.9 (65.4-66.5)
2004	68.6 (67.9-69.3)	65.9 (65.6-66.2)	55.1 (54.5-55.8)	34.5 (33.8-35.3)	29.9 (29.5-30.2)	26.2 (25.6-26.8)	79.1 (78.4-79.7)	75.9 (75.6-76.2)	66.4 (65.8-67.0)
2005	64.6 (63.9-65.3)	60.5 (60.2-60.9)	48.1 (47.4-48.8)	35.6 (34.9-36.3)	31.2 (30.8-31.5)	27.4 (26.8-28.1)	76.4 (75.8-77.1)	72.5 (72.1-72.8)	61.9 (61.2-62.6)
2006	66.4 (65.7-67.0)	62.0 (61.6-62.3)	48.2 (47.4-49.0)	35.5 (34.8-36.2)	31.0 (30.7-31.4)	27.6 (26.9-28.4)	78.0 (77.4-78.6)	73.5 (73.1-73.8)	62.3 (61.6-63.1)
2007	64.9 (64.3-65.6)	61.9 (61.5-62.3)	46.7 (45.8-47.6)	33.8 (33.1-34.4)	30.5 (30.2-30.9)	27.3 (26.6-28.1)	76.5 (75.9-77.0)	73.5 (73.1-73.8)	61.3 (60.4-62.2)
2008	62.8 (62.1-63.5)	59.0 (58.6-59.4)	43.6 (42.6-44.5)	33.3 (32.7-34.0)	30.6 (30.2-31.0)	27.5 (26.7-28.4)	75.7 (75.1-76.2)	71.8 (71.4-72.2)	59.5 (58.6-60.5)

2009	59.0 (58.3-59.7)	55.8 (55.4-56.2)	39.2 (38.2-40.2)	33.3 (32.6-33.9)	30.7 (30.3-31.1)	28.1 (27.2-29.1)	73.0 (72.4-73.6)	69.7 (69.3-70.1)	56.4 (55.4-57.4)
2010	60.8 (60.1-61.5)	58.0 (57.6-58.5)	39.4 (38.3-40.5)	34.2 (33.5-34.8)	32.0 (31.5-32.4)	29.8 (28.8-30.8)	74.8 (74.2-75.4)	71.8 (71.4-72.2)	58.5 (57.4-59.6)

* Adjusted for age, sex, race and smoking

All comparisons among 3 exposure groups have p-values <0.001 from the logistic regression models

† All comparisons among 3 exposure groups have p-values <0.001 from the logistic regression models.

‡ All comparisons among 3 exposure groups have p-values <0.001 from the logistic regression models.

Table 4. Association of pioglitazone with bladder cancer with and without adjustment for proteinuria testing

	Without Adjustment for Proteinuria Testing HR, 95% CI	**With Adjustment for Proteinuria Testing* HR, 95% CI**	**With Adjustment for Proteinuria Testing Excluding Full Urinalysis on the Same Day* HR, 95% CI**
Ever exposed to pioglitazone†	1.06 (0.87 - 1.30)	1.00 (0.82-1.22)	1.01 (0.83-1.23)
Time since starting pioglitazone#			
Less than 3.5 years	0.98 (0.76-1.26)	0.85 (0.66-1.09)	0.90 (0.70-1.15)
3.5-6.5 years	1.12(0.82-1.54)	0.99 (0.72-1.36)	1.01 (0.74-1.39)
More than 6.5 years	1.29 (0.87-1.93)	1.14 (0.76-1.70)	1.17 (0.78-1.75)
Duration of therapy#			
Less than 1.5 years	0.80(0.60-1.08)	0.69 (0.51-0.93)	0.73 (0.54-0.98)
1.5-4.0 years	1.19 (0.91-1.57)	1.05 (0.79-1.38)	1.08 (0.82-1.43)
More than 4 years	1.38 (0.97-1.96)	1.26 (0.88-1.78)	1.28 (0.90-1.82)
Cumulative dose#			
1 – 13000 mg	0.91(0.69-1.22)	0.78 (0.59-1.05)	0.83 (0.62-1.11)
13001 – 35000 mg	1.01 (0.74-1.38)	0.89 (0.65-1.22)	0.92 (0.67-1.25)
>35000 mg	1.34 (0.98-1.83)	1.21 (0.88-1.65)	1.24 (0.90-1.69)

All models are adjusted for age, sex, and year of cohort entry.

* Inclusion of testing for proteinuria in the prior 12 months. The tri-level variable was categorized as no testing, testing completed with negative test for proteinuria, and testing completed with a positive test for proteinuria

†Also adjusted for other diabetes medications

Reference group is unexposed to pioglitazone

Table 5. Evidence supporting proteinuria testing as a source of bias in studies of pioglitazone and bladder cancer

	Concept	Finding in this study
Step #1	Differential use of testing for proteinuria	Slightly more testing with pioglitazone than with other drugs and substantially more than among patients on no diabetes drugs
Step #2	Increased likelihood of positive test for proteinuria	Pioglitazone treated patients 40% more likely to have a positive test result than patients not treated with pioglitazone
Step #3	Positive test for proteinuria leads to urinalysis	Patients with a positive test for proteinuria were approximately 70% more likely to complete a urinalysis within 6 months than patients with a negative test for proteinuria
Step #4	Urinalysis following a positive test for proteinuria leads to identification of bladder cancer	Subsequent diagnosis of bladder cancer 5x more likely if urinalysis performed following a positive test for proteinuria

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