THE ASSOCIATION BETWEEN METFORMIN THERAPY AND MORTALITY FOLLOWING BREAST CANCER: A POPULATION-BASED STUDY

by

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Abstract

The association between metformin therapy and mortality following breast cancer: a population-based study

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Metformin has been associated with a reduction in breast cancer incidence, however its effect on mortality following cancer has not been adequately examined. The purpose of this study was to evaluate the impact of metformin therapy on mortality in women with breast cancer. Using Ontario health databases, this retrospective cohort examined the impact of metformin on mortality among women aged 66 years or older with diabetes and breast cancer. After a mean follow-up of 4.5 years, there was no association between cumulative metformin use and either all-cause or breast cancer-specific mortality (HR 0.97, 95% CI 0.92-1.07; HR 0.91, 95% CI 0.81-1.03 respectively per additional year of cumulative metformin use). Though metformin was not associated with a reduction in mortality in our study of older women with breast cancer, there is still a need to examine whether metformin has an effect on mortality in other breast cancer populations.
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1. Rationale

Diabetes is increasingly being recognized as a risk factor for certain cancers and has been associated with worse cancer outcomes(1). Pre-existing diabetes may increase the risk of mortality by as much as 40% in people with cancer (2). With nearly 20% of cancer patients concurrently having diabetes, this represents a large and growing population of cancer patients who are at risk of worse outcomes(3). Reasons for this disparity are attributed to lower cancer screening rates in patients with diabetes, later stage at presentation and less aggressive treatments being offered due to co-morbidities(4-6). In addition, diabetes-related comorbidities may contribute to the overall higher risk of mortality in this population(7). Diabetes is characterized by insulin resistance and compensatory hyperinsulinemia, which may also directly contribute to worse cancer outcomes. Elevated insulin levels are mitogenic and have been shown to increase tumour proliferation rates and in some settings increase the risk of cancer progression and metastases(8).

Metformin, an insulin sensitizer, is the most commonly prescribed diabetes medication. In addition to its glucose-lowering properties, recent evidence suggests that it may have anti-tumour effects (9). Two mechanisms have been proposed for metformin’s cancer-lowering effect. Indirectly, metformin may affect cancer cells through its insulin-lowering properties. By reducing glucose levels, metformin also causes a reduction in insulin levels thus reducing cancer cell growth and proliferation. Directly, metformin acts on AMPK pathways in cancer cells and inhibits downstream signaling to potent growth promoting factors (ie. mTOR, TSC2).
Several studies have evaluated the effect of metformin on cancer incidence (9, 10). However, of greater interest is the potential therapeutic role of metformin in patients with pre-existing cancer. Prior studies have shown an association between metformin use and improved prognostic indicators (11) and all-cause mortality in various populations (12-14). However, there is little evidence regarding the effect of metformin among newly diagnosed diabetic patients or its impact on cancer-specific mortality. Another important issue is the introduction of immortal-time bias in some previous studies where metformin use has been categorized at baseline based on both baseline exposure and exposure that occurred during follow-up (15, 16). Classifying drug exposure using a time-varying covariate accounts for changes in exposure that occur over time, thus minimizing this bias and allowing for more precise risk estimates (17, 18).

Given the methodological limitations of previous studies, many questions still remain as to whether metformin affects breast cancer prognosis. The goal of this study is to clarify the relationship between metformin use and all-cause and cancer-specific mortality following breast cancer in a population-based cohort of women with incident diabetes and breast cancer. To address previous analytic limitations, we examined the effect of cumulative metformin use with time-varying modeling of drug exposure.

1.1 Research Questions

The following population based study will ask two main questions regarding metformin and breast cancer mortality.

1.1.1 Primary
Among women 66 years and older with breast cancer and diabetes, what is the association of metformin therapy compared to other glucose-lowering therapies with overall and breast-cancer specific mortality?

1.1.2 Secondary

How do results from survival analyses differ when modeling drug use as time-varying exposure versus an exposure fixed at baseline?

1.2 Hypotheses

1.2.1 Primary

Metformin may have anti-tumour properties that slow tumour growth through both direct and indirect, primarily insulin-mediated, pathways. Conversely, other glucose-lowering medications that increase endogenous insulin levels have been associated with poorer cancer outcomes. We hypothesize that metformin therapy will be associated with a reduction in all-cause and breast cancer-specific mortality compared to no metformin use, and that there will be a cumulative relationship between increasing metformin use and decreased mortality.

1.2.2 Secondary

Given the risk of introducing bias when categorizing a variable as fixed at baseline when the variable changes over time during the follow-up period, we hypothesize that modeling metformin as a fixed baseline exposure will lead to an overestimation of its protective effect on survival.
2. Background

2.1 Chapter Objectives

This chapter will:

a) Provide an overview of the association between diabetes and breast cancer.
b) Discuss the evidence - epidemiologic, clinical and pre-clinical - supporting an association between metformin and cancer risk.
c) Summarize the existing literature on metformin and mortality following all cancer.
d) Review recent studies on metformin and breast cancer prognosis or mortality.

2.2 Diabetes & Breast Cancer: overview

Type 2 diabetes mellitus (diabetes) is a condition characterized by insulin resistance and hyperglycemia and affects up to 25% of the adults over age 65 in Ontario(19). Overt diabetes may be preceded by a period of 5-10 years of compensated hyperinsulinemia, during which time insulin levels are high in order to overcome the state of insulin resistance; glucose levels typical remain within the normal range during this period (20, 21). Diabetes is diagnosed once glucose levels start rising due to pancreatic beta-cell failure and decreasing insulin levels. Hyperglycemia has been associated with increased microvascular and macrovascular complications, and intensive treatment to maintain euglycemia reduces the risk of complications (UKPDS). At diagnosis, lifestyle changes that target increasing activity levels and a diet reduced in carbohydrates are recommended to maintain euglycemia and improve cardiovascular risk. If glucose remains above target, glucose-lowering medications are prescribed. Typically, insulin is reserved for maintaining glycemic control once oral glucose-lowering agents have been exhausted. The main risk factors for diabetes are age, obesity, poor diet, inactivity and genetic factors (22).
Breast cancer is the most common malignancy among women (excluding non-melanoma skin cancers), affecting 1 in 9 women during their lifetime. Over 50% of breast cancers are diagnosed in women after age 65 and incidence rises with increasing age (23). Known risk factors for breast cancer are age, genetic and hormonal factors, post-menopausal hormone use, family history of breast cancer and obesity (24). It is estimated that nearly 20% of patients with breast cancer have a history of diabetes (25).

There is evidence that patients with diabetes may in fact be at increased risk for breast cancer. In a meta-analysis of observational studies, Larsson et al. reported a 20% increased risk of breast cancer among women with diabetes (26). A more recent meta-analysis in 2011, also found that diabetes was associated with a 23% increased risk of breast cancer (27). However, two recent studies have challenged this association suggesting that detection bias may have overestimated previous results (28, 29). Both studies were carried out using health databases – one in British Columbia, the other in Denmark – and used time-varying methodology that suggested that the increase in breast cancer that occurs shortly after diabetes diagnosis is due to surveillance bias soon after diabetes is detected. Another recent study by Cheblowski et al. was also unable to show an increased risk of breast cancer among patients with diabetes (30). Current research is underway to fully understand the temporal relationship between breast cancer diagnosis and diabetes.

2.3 Insulin Resistance and Breast Cancer Risk

There are common risk factors between breast cancer and diabetes including obesity, poor diet and sedentary lifestyle (25). Even when controlling for these common risk factors between diabetes and breast cancer, the risk of breast cancer was still elevated
among patients with diabetes(31). There are also many hormones which have been implicated as mediators between obesity and cancer – estrogen, leptin, adiponectin, inflammatory cytokines – but most recent research has focused on the role of insulin and insulin-like-growth-factors (IGF) and their tumour promoting properties(8).

Insulin is a polypeptide hormone that is secreted by the pancreas in response to elevated glucose levels. It serves to regulate glucose metabolism in liver, adipose and skeletal muscle cells by binding to membrane tyrosine kinase receptors and activating downstream pathways (32). As described in the previous section, most diabetic patients are insulin resistant and thus have higher than normal circulating insulin levels. Insulin also has growth promoting effects and high levels of insulin have been shown to be mitogenic in certain cells, including breast cells(33). In addition, insulin receptors are frequently over-expressed in breast cancer cells leading them to have enhanced sensitivity to insulin’s mitogenic effects (34). Specifically it is a fetal isoform of the insulin receptor, IR-A, which is present on breast cancers cells. IR-A can either dimerize with itself or hybridize with an insulin growth factor receptor (IGF-1R) which causes activation of P13K/AKT/mTOR pathways leading to cellular growth and proliferation (33, 35), and thus high insulin levels are hypothesized to directly stimulate growth in cancer cells. Prospective clinical studies have found that women with higher endogenous insulin and e-peptide levels as well as insulin resistance at breast cancer diagnosis have higher rates of recurrence and death, suggesting that insulin may be an important prognostic factor in breast cancer(36-39).
2.4 Diabetes and Breast Cancer Prognosis

There is evidence that pre-existing diabetes is associated with worse disease-specific and overall mortality in patients with cancer (2). In breast cancer, a recent meta-analysis of 8 observational studies identified a 50% increase in all-cause mortality associated with diabetes (5). Studies since have continued to demonstrate an increased risk of all-cause mortality in breast cancer patients with diabetes (40-43). Potential explanations for these findings are multiple. First, studies have demonstrated that diabetes may be associated with lower breast cancer screening rates (4, 44) and that patients with diabetes are more likely to present with advanced stage at diagnosis (6, 45, 46). Whether the relationship between lower screening rates and advanced cancer stage is causative or whether they are independent predictors of mortality is still unclear. Second, there is also evidence that patients with diabetes are offered less aggressive cancer treatments (45, 47). This is likely related to existing comorbidities that may deter physicians from prescribing aggressive treatments such as surgery or chemotherapies due to concerns regarding complications and significant toxicities. There is also evidence that patients with diabetes may suffer more treatment related toxicities, thus compromising their dose delivery(5). Third, the role of hyperinsulinemia in promoting the growth of tumours is also an important mechanism that likely leads to the differential in mortality between diabetic and non-diabetic patients(36). In addition, there is some suggestion that patients with diabetes may develop tumours with less favourable hormone receptor profiles (30, 48). Finally, diabetes-related comorbidities may also contribute independently to the higher all-cause mortality in this population, independent of cancer.

2.5 Metformin & Cancer
2.5.1 Metformin and Cancer Risk

Metformin, a biguanide derivative, is an insulin sensitizing medication and is recommended as first line therapy in the treatment of diabetes. Metformin primarily exerts its glucose lowering effects through AMPK pathways by suppressing hepatic gluconeogenesis in liver cells. It also has an effect on AMPK pathways in muscle and adipose tissues, whereby it improves insulin resistance (49).

Metformin is a safe and well-tolerated medication with few side effects. Derived from the lilac plant, its use was first described in 1929 when it was shown to reduce glucose levels in rats. Metformin was first approved for use on humans in Canada in 1972. The most significant and commonly described side effects are gastrointestinal symptoms including nausea, bloating, diarrhea and abdominal discomfort. In clinical trials, only 5% of patients discontinue treatment due to these side effects. Lactic acidosis is a much more rare, albeit grave side effect, which occurs predominantly in patients with impaired renal function, liver or heart failure. Because of this, metformin is generally contraindicated for patients with these conditions.

Given the evidence that elevated insulin levels increase the risk of cancer, there has been much interest in the effects of insulin sensitizing agents, namely metformin, on cancer risk. There is increasing evidence that metformin use is associated with a reduction in cancer incidence. Two meta-analyses to date have summarized existing observational studies on metformin and cancer risk (9, 10). Both reviews found that metformin use was associated with a reduction in cancer risk when comparing metformin users to users of other glucose-lowering therapies.
Published in 2010, the first meta-analysis by De Censi et al. included 11 studies, 5 for all cancer-sites and 6 for cancer specific sites (breast, pancreas, prostate, hepatocellular carcinoma). They reported a 31% reduction in cancer risk among patients taking metformin (SRR 0.69, 95% CI 0.61-0.79). Three studies were identified for colon and breast cancer; but in subgroup analyses there was only a non-significant trend towards a reduction in breast or colon cancer incidence with metformin use. The studies included in the review and meta-analysis were quite heterogeneous with regards to cancer type, comparator groups, duration of diabetes and adjustment for covariates. The main concern with the studies included was their retrospective & observational nature and the risk of indication or allocation bias. Because metformin is first line therapy, metformin users may have earlier and better controlled diabetes and thus a different baseline risk of cancer than patients in the comparator groups. The issue with indication bias is particularly relevant in these studies since metformin’s effect on cancer incidence was evaluated in prevalent diabetic cohorts where the duration of diabetes varied considerably.

The second meta-analysis was published nearly two years later and included 24 studies in their review(9). Noto et al. combined data from both observational studies and randomized controlled trials (RCTs). Because of the greater number of studies included, they were also able to better describe metformin’s effect on single cancer sites. A risk estimate was reported for colorectal, hepatocellular, lung, pancreatic, breast, gastric and prostate cancers. Overall, Noto et al reported a 34% reduction in all-cancer incidence (RR 0.66, 95% CI 0.49-0.88). Among different cancer sites, metformin use was associated with a reduction in colorectal, liver and lung cancer. However, there was no statistically significant association reported between prostate, breast, pancreatic or gastric cancers. This
review confirmed findings from the earlier meta-analysis, though combining data from observational and randomized controlled trials does limit interpretation of these results due to significant heterogeneity in study design, population and analytical methods across included studies.

### 2.5.1.1 Metformin and Breast Cancer

Several studies have evaluated the specific risk of breast cancer in diabetic patients exposed to metformin. The first of these by Bodmer et al. was a nested-case control study that used the UK-based General Practice Research Databases (GPRD) to identify 305 breast cancer patients in a cohort of over 22,000 female patients with diabetes. Long-term metformin use (40+ prescriptions) was associated with a 56% reduction in breast cancer incidence (OR 0.44, 95% CI 0.24-0.82). However, short-term metformin use was not associated with risk for breast cancer in this population. The number of cases in this study was relatively small and furthermore, a prevalent diabetic cohort (> 66% of subjects had a diabetes duration greater than 2 years) was used suggesting that indication bias may have exaggerated the association seen between long-term metformin use and breast cancer risk. A study using Danish medical registries identified 393 cases of breast cancer among 4323 women with diabetes, and found that subjects who were exposed to metformin for at least 1 year had a 23% reduction in breast cancer risk (51). Similar to Bodmer et al. (50), there was no association found between metformin use and breast cancer for short term metformin duration (< 1 year); risk estimates for metformin greater than 5 years were not statistically significant, likely due to small number of subjects in this group. Recently, a large retrospective study using data from the Women’s Health Initiative Study evaluated the risk of breast cancer among women with diabetes who were metformin users versus non
users(30). Metformin use was associated with an overall lower incidence of breast cancer as compared to non-metformin use among other diabetic patients (HR 0.75, 95% CI 0.57-0.99) in analyses where metformin was modeled as a time-dependent covariate.

Some studies that examined the effect of metformin for all-cancer sites also evaluated breast cancer incidence as a secondary outcome (52-54). Two of these did not find a statistically significant reduction in breast cancer, whereas the study by Ruiter et al. which used time-dependent modeling of glucose-lowering therapies found a significant, albeit small 5% reduction in all-cause mortality per year of metformin use (HR 0.95, 95% CI 0.91-0.98)(54).

A meta-analysis specifically focused on breast cancer summarized results from seven studies, of which most have been discussed here, and found that metformin use was associated with a 17% reduction in breast cancer incidence (OR 0.83, 95% CI 0.71-0.97)(55). In sensitivity analyses, the authors found that the association between metformin and reduced breast cancer incidence was strengthened with longer duration of metformin, suggesting a dose-response relationship and thus strengthening the causal relationship between metformin and breast cancer incidence.

2.5.2 Anti-cancer properties of Metformin

Metformin has been shown to have anti-cancer properties through both direct and indirect mechanisms. The direct anti-cancer effects of metformin are postulated to be mediated via activation of AMPK that leads to downstream inhibition of TSC2/mTOR pathways. This pathway is an important cellular regulator that is involved in cell survival, protein synthesis and transcription. It is the most frequently mutated pathway in breast cancer, leading to dysregulated growth of cancer cells (8, 56). By inhibiting mTOR
pathways, metformin reduces downstream protein synthesis and thus cellular proliferation (57, 58). In activating AMPK pathways, metformin has effects that are mediated by pathways other than mTOR as well. There is evidence that p53, an important tumour suppressor gene downstream of AMPK), may be inhibited by metformin in colon cancer cells (59), but this has not been reproduced in other cell lines (60, 61).

Indirectly, metformin has anti-cancer properties via reduction in insulin levels that occurs secondarily via reduction in hepatic glucose output. High insulin levels have been associated with tumorigenesis, in part because many cancer cells express insulin receptors and are thus susceptible to increased stimulation of insulin-dependent growth-promoting pathways(33, 62).

### 2.5.3 Metformin & Breast Cancer outcomes

Several studies have looked at the effect of metformin on prognosis following breast cancer. Jiralerspong et al. first reported on a potential effect of metformin in human breast cancer patients in 2009(11). In a retrospective cohort of women being treated with adjuvant chemotherapy for early stage breast cancer, 157 patients with diabetes were identified, among which 68 were taking metformin. The authors found that metformin use was associated with a 24% greater rate of pathologic complete response (pCR) as compared to diabetic patients not exposed to metformin. In multivariable models, metformin use was associated with a 3-fold increased in rate of pCR after controlling for body mass index, tumour grade, stage, hormone receptor and human epidermal growth factor receptor 2 (HER-2) status (OR 2.95, 95% CI 1.07-8.07). Despite the improvement in pCR, there was no difference in rates of recurrence or mortality between metformin users and non-users. This study, though the first to suggest an association of metformin with breast cancer
prognosis had many limitations: the number of patients included with diabetes was small thus explaining the wide confidence intervals; diabetic patients were identified through self report and could have been misclassified; medication history was identified through pharmacy records at a tertiary care hospital but patients may have been prescribed other diabetes medications by their primary care physicians; and lastly, few diabetes related factors were included in the analysis which likely introduced confounding in the results. However, despite these limitations this study contributed to a growing body of research on metformin as a potential therapy for breast cancer.

Three recent studies have looked at the effect of metformin on markers of proliferation of breast cancer tumours in “window-of-opportunity” trials (63-65). All three studies showed a significant reduction in tumour proliferation markers among women who were randomized to metformin treatment between breast cancer diagnosis and time of surgery compared to placebo. The first of these studies was by Hadad et al. where 47 women with early stage breast cancer were randomized to metformin therapy for 2 weeks between initial core biopsy and surgery (64). In this study metformin was associated with a significant reduction in Ki67, a predictor for clinical and pCR in the neo-adjuvant setting, as well as gene expression of other markers of tumour proliferation. In the largest study where 200 women were randomized to metformin, a decrease in the tumour marker Ki67 was observed in patients who had an accompanying reduction in insulin resistance suggesting an insulin-driven mechanism for metformin’s anti-cancer properties (63).

However, in this study metformin therapy was stopped 3 days prior to surgery and thus only the residual effects of metformin could be assessed. Most recently, Niraula et al. (65) confirmed these previous findings in a smaller study that reported a significant reduction in
both Ki67 and a marker of tumour apoptosis. Together, these three studies support the need for ongoing evaluation of metformin in the clinical breast cancer setting.

2.5.4 Metformin & Cancer Mortality

There are many studies that have examined the effect of metformin therapy on mortality following cancer (table 1). To summarize this literature we conducted a systematic review and meta-analysis of studies that evaluated the effect of metformin therapy on mortality following cancer (Lega IC et al. 2012 unpublished). Our initial search in EMBASE and Medline database conducted in May 2012 yielded 692 articles. After applying our eligibility and exclusion criteria, our final sample included 9 studies to be included in our systematic review and meta-analysis (12-16, 66-69). We searched for both observational studies and controlled clinical trials, but only retrieved observational studies from our search. We limited our review to studies that were conducted in patients with diabetes and any cancer. The primary exposure in all studies was exposure to metformin therapy and the primary outcome was all-cause mortality.

Nine observational studies were analyzed in the meta-analysis: one study included all cancers, two included breast cancer (one in women with HER2+ disease, one in women with triple-negative receptor disease), two included colon cancer, and one each included pancreatic, prostate, liver and ovarian cancer patients. The majority of studies categorized metformin use as ever/never exposure based at time of cancer diagnosis. For example, if a patient was currently on metformin at the time of their cancer diagnosis, they were categorized as ‘ever’ being exposed to metformin; conversely patients who were not on metformin at the time of cancer diagnosis were considered ‘ever’ exposed. Two studies (15, 16) defined a fixed ever/never exposure to metformin based on exposure which occurred
prior to or *during* the follow-up period, and one study classified metformin exposure as ever/never in the three months preceding breast cancer diagnosis (12).

The main finding of our systematic review and meta-analysis is that all but two of the studies reported a reduction in mortality associated with metformin therapy with adjusted hazard ratios (HR) that varied between 0.23 and 0.85. Only two studies, one in patients with triple-negative receptor breast cancer and the other in ovarian cancer patients, showed no effect (66, 68). In pooled analyses, exposure to metformin was associated with a 33% reduction in all-cause mortality (pooled adjusted HR 0.67, 95% CI 0.61-0.73, $I^2=0\%$) (figure 1). This association remained robust in sensitivity analyses that reduced the risk of bias by only including studies that adjusted for cancer stage at diagnosis, a strong predictor of mortality and a potential confounder (pooled HR 0.66, 95% CI 0.53-0.71).

From our systematic review of the literature, we were able to show that metformin use at time of cancer diagnosis is associated with a significant reduction in mortality. However, despite these findings the clinical significance of our results is limited by the observational nature of the included studies, biases, as well as by diverse clinical populations. The main methodological limitations of the studies were 1) risk of indication bias given the fact that prevalent diabetic populations were studied and 2) categorization of metformin as ever/never use at baseline and in some cases, risk of immortal time bias. These issues, as well as limitations of the existing literature in general will further be discussed in the chapter “Methodological concerns with the existing literature”.

2.5.4.1 Metformin and Mortality following Breast Cancer

Three studies have looked specifically at the effect of metformin on mortality following breast cancer. The results from these studies are not consistent, likely because the
study population and methodology differed among studies. He et al. found that metformin therapy was associated with improved all-cause survival among patients with human epidermal growth factor receptor 2 (HER2+) breast cancer (HR 0.52, 95% CI 0.28-0.97) (16). HER2 is a growth factor that is involved in downstream signaling via AMPK/mTOR; the authors chose this subgroup because metformin has been shown to decrease HER2 expression by inhibiting mTOR signaling in vitro. In contrast, Bayraktar et al. did not find an association between metformin and mortality in a population of women with triple negative receptor breast cancer, a breast cancer subgroup with poorer prognosis in general (66). Both studies were single institution, observational studies that defined metformin use at time of breast cancer diagnosis. In terms of cancer covariates, both accounted for stage and receptor status at diagnosis as well as clinical covariates such as body mass index and menopausal status. However the heterogeneous breast cancer populations make comparisons between these two studies difficult.

The study by Currie et al. looked at the effect of metformin on all cancers, but had a large subgroup of 1182 women with diabetes and breast cancer that were evaluated separately in a subgroup analysis of a larger study of all cancers (12). When comparing metformin users to both diabetic patients on other glucose lowering therapy and non-diabetic patients, this study did not find an association between metformin use and mortality following breast cancer (HR 0.97, 95% CI 0.70-1.35) (12). The main limitation of this study is that it combined both diabetic and non-diabetic users in the comparator group, with a large proportion of the comparator group being made up of non-diabetic patients (over 23,000 non-diabetic women with breast cancer). Because diabetes alone has been associated with a worse prognosis among breast cancer patients, keeping both in the
comparator group can lead to biased results and does not allow for a conclusion to be made regarding drug effects versus diabetes effects.

2.5.5 Methodological concerns with existing literature

In summarizing the above literature we must keep in mind the importance of adequately accounting for bias, especially when conducting observational research. Interpretation of previous studies is hampered by several methodological inconsistencies, such as inclusion of prevalent diabetic populations with varying severity and disease duration, differing durations and timing of metformin exposure, heterogeneity of cancer populations and minimal accounting of cancer treatment-related factors. More specifically, the two main biases that need to be considered are indication bias and immortal time bias (see table 2). The risk of indication bias is accentuated when using a prevalent diabetic cohort with patients of varying diabetes duration. Since metformin is first line therapy and often reserved for patients with better glycemic control, patients who are prescribed metformin may have earlier, less severe disease and may therefore have lower all-cause mortality possibly due to more aggressive cancer treatment and/or lower diabetes-related comorbidity(6). One way to reduce indication bias in a prevalent diabetic cohort is to use a propensity score (70). The propensity score determines the probability of being assigned to a treatment (or exposure) based on observed baseline characteristics and is estimated using a logistic regression model(71). Another way to circumvent indication bias is to use an incident diabetic cohort where all subjects are followed from a similar point in their diabetes history. The indication for starting a glucose-lowering drug should therefore be more similar in this context, and metformin use is less likely to be a marker of recent versus long standing diabetes.
Immortal time bias, often referred to as survivor-treatment or time-dependent bias, also needs to be carefully accounted for in research studies that include an exposure that varies over time (see table 2). Immortal time bias occurs when an exposure is classified at the start of follow-up, though it varies over time and may not even have been present at the beginning of follow-up. This causes exposures to be misclassified as being exposed during periods of unexposed time. The unexposed period prior to drug exposure is considered ‘immortal’, since by definition, the subject who is treated/exposed cannot have died prior to receipt of treatment or exposure. Incorrect consideration of this period of immortal time leads to immortal-time bias that can overestimate the benefit of treatment because patients who live longer have more opportunity to have the exposure. The time prior to when metformin is used is categorized as ‘ever being exposed’, rather than being treated as a variable whose value changes over time (18, 72). Immortal-time bias can be circumvented by correctly accounting for the time-varying nature of exposure/treatment. This can be done by setting up the data to be analyzed in a counting process format in which the dataset contains more than one record per subject and where each record represents a pre-specific time interval allowing for exposures to be updated at each time interval and to be analyzed as time-varying covariates (73).

Overall, findings from both epidemiological and window-of-opportunity studies suggest an association between metformin and breast cancer prognosis, as well as mortality. Past studies have been limited by methodological inconsistencies as well as heterogeneous study populations. The current study aimed to clarify the relationship between metformin use and all-cause and cancer-specific mortality following breast cancer in a population-based cohort of women with diabetes and breast cancer. To address
previous analytic limitations, we examined the effect of cumulative metformin use using time-varying modeling of drug exposure in an incident diabetic population.
3. Methods:

3.1 Chapter Objectives

This chapter will:

a) Describe the study design and data sources used in this study.
b) Describe how the study cohort was defined.
c) Define study variables including exposure, outcomes, and other covariates.
d) Describe the analysis plan, including the modeling of time-varying covariates.

3.2 Design

We conducted a population-based cohort study to study the effect of metformin therapy on all-cause and breast cancer-specific mortality among women with diabetes diagnosed after age 66 and who were subsequently diagnosed with breast cancer. We used anonymized, administrative healthcare databases in Ontario, Canada from April 1, 1997 and March 31, 2008. Databases are individually linkable through anonymous identifier key numbers (ikn – an encrypted version of the patient health insurance number) that allow information from different databases to be gathered for individual patients.

3.3 Subjects

3.3.1 Inclusion criteria

The study population was identified from a cohort of women aged 66 or older between April 1, 1997 and March 31st, 2008 who had a new diagnosis of diabetes in the Ontario Diabetes Database (ODD) (described below) occurring after age 66 years. We limited our cohort to women age 66 years or older in order to have a cohort of newly diagnosed diabetic patients in whom we could account for all glucose-lowering drug exposure from time of diabetes diagnosis. From there, our study population included all women with a new diagnosis of diabetes after age 66 who subsequently developed breast
cancer, as identified from the Ontario Cancer Registry (OCR) (described below). Date of cohort entry was defined as date of breast cancer diagnosis (figure 2).

3.3.2 Exclusion criteria

We excluded all women who were diagnosed with diabetes after their breast cancer diagnosis, so that all women had preexisting diabetes at time of breast cancer diagnosis. Second, women who had a previous history of any cancers, excluding non-melanoma skin cancer, prior to breast cancer diagnosis were also excluded. Third, we excluded women who had a date of last health care more than 365 days from our study end of follow-up, yet who were coded as still being alive in March 2010. Given the age and comorbidities of this population, it is unusual that these patients would have not accessed the health care system for one year and we could not ascertain whether these patients had moved from the province, or had died and not been appropriately coded in the Registered Persons Database (RPBD).

3.3.3 Diabetes cohort

Diabetes status was determined by linking subjects to the validated ODD an administrative database registry of diabetes patients. Subjects are included in the ODD if they either have 1 hospitalization or two physician service claims with a diagnosis of diabetes within a 2 year period. Data regarding hospitalizations are derived from hospital discharge abstracts prepared by the Canadian Institute for Health Information (CIHI) Discharge Abstract Databases (DAD). Discharge abstracts contain 16 possible diagnostic fields and discharge abstracts which contain an International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) code of 250.x on any one of those fields signifies a patient with either pre-existing diabetes or diabetes diagnosed in hospital. From
2002 onwards, discharge abstracts use ICD10 codes. Physician claims submitted to the Ontario Ministry of Health Ontario Health Insurance Program (OHIP) must bear the diagnostic code ‘250’. This registry has been validated against primary care charts and shown to have a high sensitivity (86%) and specificity (97%) for identifying individuals clinically diagnosed with diabetes(74). Though the ODD does not allow us to distinguish type 1 and type 2 diabetes, new onset type 1 diabetes is rare after age 65 years and thus there was little risk of misclassifying a patient with type 1 diabetes who in fact had type 2 diabetes.

Our diabetes cohort was limited to subjects who were diagnosed with diabetes after age 66 years. This was done for two reasons. First, to ensure an inception cohort, subjects were identified at a common stage of their diabetes. Second, because drug exposure can only be universally captured through the Ontario Drug Benefit (ODB) program for individuals aged 65 years or older, by limiting our cohort to patients older than 66 years we were able to capture exposure to all glucose-lowering medication, including exposure in the year prior to diagnosis. Metformin can be used in “pre-diabetes” and so we hypothesized that some prescriptions for metformin could have preceded the diagnosis of diabetes.

3.3.4 Cohort entry (breast cancer diagnosis) and follow-up

See figure 2 for study timeline. Our cohort was limited to women diagnosed with breast cancer after diabetes diagnosis. Cohort entry was defined by a new diagnosis of invasive breast cancer within our incident diabetes cohort, determined through ICD9 codes (174) in the Ontario Cancer Registry (OCR). The OCR does not capture data on non-invasive breast cancers (i.e. carcinoma in situ). The OCR maintains records on all cancers (excluding non-melanoma cancers) diagnosed in Ontario since 1964 and derives cancer
data from four sources: hospital discharge summaries, pathology reports, death certificates and clinical records from cancer centers. The OCR has been shown to have a completeness rate of 95% for capturing all cancers in Ontario (75). Moreover an estimated 83% of all cancer cases have been microscopically verified, with a higher rate among breast cancer cases, indicating a good level of accuracy (76).

The study period was from April 1, 1997 to March 31, 2010. Women were followed from time of breast cancer diagnosis until death or March 31, 2010, whichever came first.

3.4 Drug exposure

The main exposure of interest was metformin use. We identified all drug prescriptions, including metformin, using the Ontario Drug Benefit (ODB) database, which contains information on all publicly funded prescriptions dispensed to Ontario residents aged 65 years or older. We captured all glucose-lowering drug exposure from the time of diabetes diagnosis until the end of follow-up. We thus took into account drug exposure that had occurred prior to breast cancer diagnosis, i.e. between diabetes diagnosis and breast cancer. We classified glucose lowering drug exposures into the following categories: metformin, sulfonylureas (glyburide, gliclazide, glibencamide, repaglinide), thiazolidinediones (rosiglitazone, pioglitazone) and insulins (Glargine, Detemir, Humalog, Lispro, NPH, R). Given the small number of patients on other anti-diabetic medications we did not classify exposure to these other drugs.

For drug exposure that happened prior to breast cancer diagnosis, we classified baseline exposure to each of the 4 drug categories as never/ever depending on whether or not a subject had at least one drug prescription dispensed in the ODB at any time between
diabetes diagnosis and cancer diagnosis. Drug exposure that occurred after breast cancer was our primary exposure of interest. We calculated cumulative duration of total use of each glucose lowering drug from time of cancer diagnosis to end of follow-up period. The value of this variable was defined for each day during follow-up, so that its value was allowed to change as total duration of exposure changed over the course of follow-up. The cumulative exposure variable was derived from the date of the prescription and the number of days supplied for each prescription filled.

3.5 Outcomes

3.5.1 Primary outcome

The primary outcome was all-cause mortality based on records from the Registered Persons Database (RPDB), which contains demographic and residential data, as well as death records from death certificates. The RPDP does not include information on cause of death.

3.5.2 Secondary outcome

The secondary outcome was breast cancer-specific mortality and was based on death records from OCR. The OCR defines deaths as being cancer-specific, based on ICD 9 or ICD 10 codes, or non-cancer related. A validation study found that the OCR had a high sensitivity (95%) and high specificity (88%) for defining breast cancer-specific mortality (77). Cause of death databases at the OCR are updated yearly, but have a 3 year lag from the present. Because of this, our follow-up for our secondary outcome was until December 31st, 2008.
3.6 Covariates

3.6.1 Demographic variables

Baseline demographic data included age, rural vs. urban location and income, as a indicator of socioeconomic status. Age was recorded from the Registered Persons Database (RPDB). The RPDB was also used to indirectly assess income status through postal codes. Though direct income information cannot be derived through Ontario databases, household income levels can be described through postal code conversion files that then divide the population into income quintiles. Lastly, postal codes were also used to define urban versus rural location.

3.6.2 Comorbidity

Co-morbidity score was estimated using the John Hopkins Adjusted Clinical Group (ACG) case-mix. The ACG system uses both ambulatory and in-patient health care data and is an important risk adjustment tool in populations that are unlikely to have had hospitalizations. The ACG uses recorded ICD9 codes from outpatient visits over the year prior to cohort entry and records comorbidity by specific disease category and provides composite categories such as unstable or stable chronic diseases (78). Each ICD code is assigned one of 32 distinct diagnostic clusters known as Aggregated Diagnosis Groups (ADG). Though the initial ACG/ADG system was used to record health care utilization, it has been validated as being predictive of 1-year mortality in an ambulatory setting of patients with diabetes in Ontario (79). In order to model the ADG categories, we used a system developed by Austin et al. where different “weights” are assigned to conditions and used to arrive at a numeric score that can predict mortality (80).
Specific co-morbidities were also determined at baseline and derived from CIHI and OHIP databases. We also performed a 1-year look-back to identify subjects who had a history of cardiovascular disease, chronic obstructive pulmonary disease (COPD) and/or hypertension. See table 4 for ICD9 and ICD10 codes used for these diagnoses.

3.6.3 Breast Cancer Treatments

In defining our breast cancer cohort, we did not have data on breast cancer stage at diagnosis, or hormone receptor status (i.e. ER, PR, HER2). However, we did have data on four important breast cancer treatment variables: surgery, radiotherapy, chemotherapy and hormonal therapy.

All breast cancer treatment variables were captured during the first 365 days following breast cancer diagnosis. The surgery variable was the only exception, where it was defined between 4 months prior to, and 365 days following breast cancer diagnosis. Given how data is recorded at the OCR, it is plausible that a patient’s date of surgery could follow their OCR diagnosis date as some diagnoses are made prior to surgery based on biopsy specimens.

Surgery for breast cancer was identified based on procedure codes from the CIHI DAD related to breast surgery(81). We included all procedures relating to both radical and conservative breast surgery. Information regarding radiotherapy and chemotherapy was derived from physician billing claims submitted to the Ontario Health Insurance Program (OHIP). For the chemotherapy variable, we were not able to determine whether chemotherapy was prescribed as primary, adjuvant treatment or whether it was given for metastatic disease. To define radiotherapy, we used two definitions based on whether radiotherapy occurred before or after 2002. Prior to 2002 there were no OHIP planning
codes for radiation so we used an algorithm that is based on OHIP billing fee codes. For a patient to be defined as having received radiation, the following criteria has to be fulfilled: 1) billing code for new consultation with a radiation oncologist; 2) billing code for assessment/reassessment within 6 months of initial consultation; and 3) 2 more billing codes for assessment/reassessment within the 5 next weeks. After 2002, the Ministry introduced fee codes for radiotherapy planning, and so those could be used after 2002 to identify patients undergoing radiotherapy (table 5).

Exposure to hormonal therapy (aromatase inhibitors and tamoxifen) within the first year following breast cancer diagnosis was obtained from prescriptions dispensed in the ODB. We classified hormonal therapy as never/ever in the year following breast cancer diagnosis for both aromatase inhibitors and tamoxifen. We did not take into account switches from one class to the other.

3.7 Statistical Analysis

3.7.1 Drug exposure modeling

Drug exposure to glucose-lowering drugs was defined and modeled in three different ways. For our primary analysis, we calculated the cumulative duration of past exposure to glucose-lowering medications for the time period between cancer diagnosis and death or end of follow-up. This variable was updated after each day of follow-up, thereby reflecting the accurate cumulative dose of a glucose-lowering medication that a patient had been exposed to throughout the follow-up period.

In secondary analyses, we categorized metformin in two different ways. First, we classified glucose-lowering drugs as a time-varying never/ever exposure. This meant that
subjects were coded as ‘never’ or ‘ever’ exposed to a drug based on having at least one prescription from breast cancer diagnosis to the end of follow-up. A subject became ‘ever’ exposed on the date on which the prescription was filled, and remained ‘ever’ exposed until the end of the follow-up period. If a subject was never exposed to the drug at any point in the follow-up period, they remained ‘never’ exposed. Second, to illustrate the effects of immortal time bias, we classified metformin use as fixed never/ever exposure based on exposure at any time in the follow-up period. If a patient had filled one or more prescriptions for metformin at any time between cancer diagnosis and end of follow-up they were coded as ‘ever’ being exposed to metformin, and this remained fixed for the entire follow-up period. The fixed never/ever method did not take into account any changes in glucose lowering therapy that occurred during the follow-up period.

3.7.2 Counting Process Format

In order to model drug exposure as a time-varying covariate, the data were set up in counting process format(73). Most commonly, data are set up such that there is one line of observations for each subject. To create the counting process format, each record in the dataset represented one day of follow-up for each individual. For example, if a patient was in the study for 2 years (712 days), there would be 712 records for that patient. This was done so that drug exposure could be updated daily. Time since breast cancer diagnosis was used as the underlying time scale for the Cox proportional hazard model. Two separate datasets were set up in order to model each outcome, given the different follow-up times for all-cause mortality and breast-cancer specific mortality.

3.7.3 Analysis
All analyses were performed using SAS software (version 9.2). Survival analysis was performed using a Cox proportional hazard model to estimate the association of metformin use with survival following breast cancer. We first performed univariate analyses on the main exposure and all covariates to assess their effect on mortality. Multivariable analyses were then performed. We retained covariates that changed the risk estimate by > 10% or those that were clinically relevant. Finally we performed multivariable Cox proportional hazard models to determine the effect of metformin on survival after adjustment for potential confounders. We adjusted for the following variables in our main analysis: glucose lowering drug at baseline (i.e. at time of breast cancer diagnosis) (metformin, SU, TZD and insulin), cumulative use of glucose lowering drugs (SU, TZD and insulin), age at breast cancer diagnosis, duration of diabetes, year of cohort entry, weighted ACG score, breast cancer treatments within first year of diagnosis (surgery, chemotherapy, radiotherapy, hormonal therapy). For the fixed never/ever analysis, the model was run on a simple dataset that contained one observation for each subject and in which all covariates were fixed and defined at baseline.

For our primary analysis, we expressed the hazard ratio per additional year of cumulative use of metformin, by multiplying the parameter estimate (obtained per day of use based on the construction of the dataset) by 365. For our secondary analyses, HR was expressed for ever (vs. never) exposure to metformin. In the first sensitivity analysis metformin exposure was modeled as a time-varying exposure; whereas in the second model, it was fixed at baseline.

Given the nature of time-varying covariates we were not able to assess for statistical collinearity between our main exposure and included covariates.
Various sensitivity analyses were performed to explore our results. First, because there is controversy as to whether sulfonylureas and insulin may worsen cancer prognosis and the effect of metformin may only be protective in comparison, we limited our cohort to metformin monotherapy users and diabetic patients on no drug therapy. Second, given the potentially harmful effects of insulin as well as its role as an effect modifier, we stratified our cohort based on insulin users and non-users. Lastly, in an attempt to exclude women with advanced stage disease or those too ill to be appropriately treated for their breast cancer, we limited our cohort to only include women who had surgery as a baseline treatment.

**Ethics**

This project was approved by the institutional review board of Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.
4. Results

4.1 Chapter Objectives

This chapter will:

a) Describe cohort characteristics.
b) Summarize results from the primary and secondary analyses.
c) Assess the robustness of estimates through sensitivity analyses.

4.2 Baseline characteristics

The study population consisted of 2361 women with incident diabetes and breast cancer (table 6). Mean age at breast cancer diagnosis was 77.4 (standard deviation, SD +/- 6.3) years, and women had a mean duration of diabetes of 3.6 years (SD +/- 3.0) at time of breast cancer diagnosis. Mean follow-up from time of breast cancer diagnosis was 4.5 years (SD +/- 3.0) for all-cause mortality, and 3.7 years ( +/- 2.8) for breast cancer-specific mortality. During the study period, 1094 (46.3%) patients were prescribed metformin, 284 (12.0%) were prescribed glucose-lowering medications other than metformin (i.e. insulin, sulfonylureas or TZDs) and 983 (41.6%) were not taking any glucose-lowering medications. Among metformin users, 818 (34.7%) were also exposed to sulfonylureas, 142 (6.0%) to TZDs and 261 (11.1%) to insulin. Overall, there were 1101 (46.6%) deaths and 386 (15.1%) breast cancer-specific deaths in the cohort.

To describe baseline variables by drug exposure, we categorized women based on prescriptions filled at any time during the follow-up into users of metformin (+/- other drugs), users of other glucose-lowering drug but not metformin, and no medications (table 6). Metformin users were younger at breast cancer diagnosis and had longer duration of follow-up compared to both non-metformin groups. Mean duration of metformin therapy
after cancer diagnosis was 2.9 years (±/− SD 2.3). Metformin users were less likely to have comorbidities compared to the non-metformin group receiving other glucose-lowering medications, but the baseline prevalence of comorbidities was similar between metformin users and non-treated diabetic patients. Metformin users were more likely to have surgery and radiation as primary treatments of their breast cancer, while subjects treated with other glucose-lowering drugs were the least likely to receive these treatments. Aromatase inhibitors were more likely to be prescribed to metformin users, whereas tamoxifen use was most common among the group prescribed glucose-lowering therapies other than metformin. Other baseline characteristics were similar between treatment groups (table 6).

### 4.3 Primary analysis: Time-varying drug exposure

In our primary analyses, when modeling cumulative duration of metformin exposure between breast cancer diagnosis and end of follow-up as a time-varying covariate there was no association between metformin exposure and either all-cause or breast cancer-specific survival (HR 0.97, 95% CI 0.92-1.02 and HR 0.91, 95% CI 0.81-1.03 for each additional year of cumulative past metformin exposure) (table 7). When the association of five years of cumulative metformin exposure was estimated from our data we found a HR 0.86 (95% CI 0.68-1.08) with all-cause mortality and HR 0.65 (95% CI, 0.35-1.07) with breast cancer-specific mortality.

In terms of other glucose lowering medications, TZD use was associated with a significant increase in breast cancer-specific mortality (HR 1.52, 95% CI 1.02-2.26 per additional year of cumulative past exposure) but not all-cause mortality (HR 1.06, 95% CI 0.75-1.27). Conversely, exposure to sulfonylureas after cancer diagnosis was associated
with a significant reduction in all-cause mortality (HR 0.95, 95% CI 0.89-0.99 per additional year of cumulative past exposure), but there was no association with sulfonylurea use and breast cancer-specific mortality (HR 0.91, 95% CI 0.80-1.02). There was no association between insulin use and either all-cause or breast cancer-specific mortality.

In terms of exposure to glucose-lowering therapy prior to breast cancer diagnosis, only exposure to sulfonylureas prior to breast cancer diagnosis was a significant independent predictor of both higher all-cause and breast cancer-specific mortality (HR 1.35, 95% CI 1.15-1.58 and HR 1.42, 95% CI 1.10-1.82 for ever use), while there was no association with prior use of other glucose-lowering agents (table 7b).

### 4.4 Secondary analyses: never/ever categorization of exposure

In our secondary analysis whereby drug exposures were categorized as never/ever time-varying covariates, metformin treatment was not associated with either all-cause or breast specific-survival (HR 0.93, 95%, 0.78-1.10 and HR 0.92, 95% 0.70-1.21) (table 8). In this model, exposure to insulin during follow-up was associated with a greater than two-fold risk of all-cause mortality and a 36% increase risk in breast cancer-specific mortality (HR 2.08, 95% CI 1.66-2.61 and HR 1.98, 95% CI 1.29-3.03). These results may reflect protopathic bias since many patients may be switched to insulin in the months prior to dying due to disease progression.

When we categorized drug exposure as never/ever fixed covariates, metformin exposure was associated with a 40% reduction in all-cause mortality and a 47% reduction in breast cancer-specific mortality (HR 0.60, 95% CI 0.52-0.69 and HR 0.53, 95% 0.42-0.67) (table 8). Ever being exposed to TZD was also associated with a significant reduction
in all-cause mortality (HR 0.63, HR 0.45-0.89). Insulin and sulfonylurea use was not associated with either all-cause or breast cancer-specific mortality.

4.5 Sensitivity analyses

We performed the following sensitivity analyses to test the robustness of our findings. First we limited our cohort to metformin monotherapy users and to patients on no glucose-lowering agents since these two groups should be clinically most similar in terms of diabetes duration and severity. Second, we excluded all insulin users given the concern that insulin use may worsen prognosis and effectively cancel a positive effect of metformin. Finally, in an attempt to restrict our cohort to women with earlier stage disease, we only included women who underwent a surgical procedure within the first year following breast cancer diagnosis.

Overall, there was no association between cumulative metformin therapy and mortality in any of the sensitivity analyses (table 9-11). When limiting the cohort to metformin users and the non-therapy group (defined as no glucose-lowering therapy during follow-up after breast cancer diagnosis), similar to the primary analysis, sulfonylurea use prior to breast cancer diagnosis was significant associated with both an increase in all-cause and breast cancer-specific mortality (HR 1.96, 95% CI 1.53-2.51, and HR 2.16, 95% CI 1.50-3.10 respectively) (table 9). Prior insulin use was associated with an increase in all-cause mortality (HR 2.51, 95% CI 1.33-4.73) but not cancer-specific mortality (HR 1.48, 95% CI 0.45-4.87).

When excluding all insulin users from the cohort, similar to our main analysis, we found a small but significant reduction in all-cause mortality with cumulative sulfonylurea
use (HR 0.93, 95% CI 0.88-0.98), however there was no association with breast cancer-specific mortality (HR 0.95, 95% CI 0.85-1.06). Sulfonylurea use prior to breast cancer diagnosis was the only glucose-lowering therapy associated with an increase in either all-cause or breast cancer-specific mortality (HR 1.47, 95% CI 1.24-1.73 and HR 1.27, 95% CI 1.00-1.61 respectively) (table 10).

Finally, results were overall similar in sensitivity analyses where the cohort was limited to women who had undergone primary surgery for their breast cancer (table 11).
5. Discussion

5.1 Chapter Objectives

This chapter will:

a) Summarize results from our study.
b) Contrast findings with results from previously published studies.
c) Explain the methodological strategies employed to minimize bias.
d) Discuss the biological plausibility of metformin having an effect on mortality following cancer.
e) Discuss clinical and research implications of this study, as well as future directions.

Our study is the first to evaluate the association of cumulative metformin use with mortality following breast cancer, using various analytic approaches. When modeling metformin use as a cumulative, time-varying exposure there was no association between past metformin use and mortality. In secondary analyses, where metformin exposure was modeled as time-varying never/ever exposure, there was no association between metformin use and mortality. However, when metformin exposure was treated as a fixed never/ever variable based on baseline exposure or exposure that occurred during follow-up, metformin was associated with a significant reduction in mortality. These contrasting findings illustrate the complexities of modeling drug exposure in survival analyses. The apparent benefit observed in the latter analysis likely represents an overestimation of metformin’s effect due to immortal time biases.

5.2 Major findings

5.2.1 Primary objectives

This large, population-based study of older breast cancer patients with diabetes did not find an association between total cumulative duration of past metformin therapy and
mortality. Of note, our findings do suggest a 9% reduction in breast cancer-specific mortality per additional year of cumulative metformin use that was not statistically significant, which translates into a potential 38% decrease over 5 years (HR 0.62, 0.35-1.07). Given the small number of women with long-term metformin exposure (n=150 subjects and shorter mean follow-up period for breast cancer-specific mortality (3.7 years), lack of power and cannot be excluded as a reason for our failure to detect statistical significance. Further studies in larger populations are needed.

In terms of other glucose-lowering medications during the follow-up period, we also found that one year of cumulative sulfonylurea use was associated with a small, albeit statistically significant improvement in overall survival (HR 0.95, 95% CI 0.89-0.99). Given that both the risk estimate and the upper limit of the confidence interval abuts one, we do not believe this finding to be of clinical significance. Another notable finding was that cumulative TZD use was associated with a significantly increased risk of breast cancer-specific mortality, though there was no effect on overall mortality (HR 1.52, 95% CI 1.02-2.26). These findings may be due to confounding since TZD medications during the study period were likely prescribed to persons failing first line drugs (metformin, sulfonylurea) or to patients who had a contraindication to them due to comorbidities such as renal or cardiac impairment. Thus, these patients may also have been more likely to have advanced breast cancer and a worse prognosis. Though TZD use has not been directly associated with breast cancer risk or prognosis in epidemiological studies, there is pre-clinical evidence that peroxisome proliferator-activated receptor (PPAR) activation by TZD may modulate breast carcinomas (82). Furthermore, there is mounting evidence that TZDs are associated with a
significant increase in risk of bladder cancer which suggests that there may be an underlying mechanism for carcinogenesis with TZDs (83).

5.2.2 Secondary objectives

This study also assessed the effect of modeling metformin as a never/ever time-varying covariate and as a fixed never/ever covariate defined at baseline according to use during the follow-up period. When metformin was modeled as a never/ever time-varying exposure there was no association between metformin use and either breast cancer-specific or all-cause mortality. Similarly, there was no association between sulfonylurea or thiazolidinediones use and mortality. However, insulin use was associated with a 2-fold increase in both all-cause and breast cancer-specific mortality in never/ever time-varying analyses (HR 2.08, 95% CI 1.66-2.61 and HR 1.98, 95% CI 1.29-3.03 respectively). This finding was not replicated in our main analyses and may have been confounded by protopathic bias, bias that occurs when a treatment for the first symptoms of a disease/outcome is interpreted as causing the outcome (table 2). For example, as a patient develops more advanced cancer or is nearing death, their glucose control may worsen leading to insulin being prescribed. Insulin may thus be a marker of worsening control due to advancing cancer and these results should be interpreted with caution. Furthermore, these findings illustrate the risk of bias when evaluating the role of insulin therapy and cancer risk. Though observational studies have suggested a link between insulin and cancer, the role of bias in these studies cannot be excluded (84-86).

When metformin exposure was categorized as a fixed covariate at baseline based on exposure that occurred either at baseline or during the follow-up period, women who ever used metformin had a significant decrease in both all-cause and breast cancer-specific
mortality (HR 0.60, 95% CI 0.52-0.90, and HR 0.53, 95% CI 0.42-0.67 respectively). Ever being exposed to thiazolidinediones was also associated with a reduction in all-cause mortality (HR 0.63, 95% CI 0.45-0.89), however there was no association with breast cancer-specific mortality. There was no association between ever exposure to insulin or sulfonylurea and either outcome. Glucose-lowering therapy prior to cancer diagnosis (not including baseline exposure) was not included in this model since there was a significant correlation coefficient between these variables and the main exposure variables. These findings likely reflect the effect of immortal-time bias.

5.3 Limitations and strengths

5.3.1 Limitations

Findings from our study should be interpreted in the context of the following limitations. First, we did not have access to laboratory data and therefore were unable to assess extent of glycemic control in our subjects. Poor glycemic control is a predictor of mortality in patients with diabetes (87). We attempted to address this issue by constructing an incident diabetic cohort where patients had similar duration of diabetes and thus would be more likely to have similar glycemic control.

Another important limitation is that we did not have access to important clinical breast cancer predictors such as stage at diagnosis, receptor status and more detailed information about treatments received. Including these variables would have been helpful since they are predictors of survival in this population. In general, patients with diabetes are less likely to receive aggressive treatments for breast cancer and are more likely to present with later stage disease as compared to non-diabetic patients(5). However, we do not know how these predictors differ among diabetic patients treated with different glucose-lowering
therapies. In our study, patients exposed to metformin were more likely to undergo surgery and radiotherapy, suggesting that metformin users may have a better prognosis. Despite this, we did not find an association between cumulative metformin use and mortality; this does not lead us to suspect that our results were biased since a healthy user effect would tend to bias the results positively away from the null.

Third, our study was conducted in an elderly cohort of women who were at least 66 years of age or older at the time of diabetes and breast cancer diagnosis. Given this our findings are not generalizable to younger breast cancer patients, nor to patients with long-standing diabetes. Though over 45% of breast cancers are diagnosed in women after age 65 years, women who develop both diabetes and breast cancer at a later age may have different disease biology, such as less severe insulin resistance and metabolic factors driving their cancer risk (88). Metformin’s effect in such a population may be lower, and its effect may be modest when compared to other interventions. While tumour characteristics have not been shown to differ greatly between younger (age < 80 years) and older postmenopausal women, breast cancer treatments may differ quite substantially between these two age groups. Older women may be less likely to receive aggressive therapy for their breast cancer and are more likely to die from their disease (89, 90). Since over 30% of our cohort was over age 80 years, this may explain why we did not find an effect for metformin. Another possibility is that metformin’s anti-tumour effect may either be limited to early stage breast cancer or have a synergistic effect with chemotherapy, as in Jiralerspong’s study population (11). Fourth, whether or not metformin has an effect in non-diabetic breast cancer patients also remains to be seen – our findings are only generalizable to women with diabetes and breast cancer. Whether there is an association between
metformin and mortality following breast cancer in non-diabetic patients is currently being addressed in a multi-center randomized trial (90).

Finally, our mean follow-up period of 4.5 years was relatively short and a longer exposure to metformin may be required to detect an association of metformin with survival, especially where five-year survival following breast cancer is as high as 88%(91). However, compared to He and Bayraktar our mean follow-up is similar (4.0 and 5.2 years months respectively)(16, 66). Future studies should attempt to follow patients for a longer time to ensure that there is no effect of metformin on mortality after a duration greater than 5 years.

5.3.2 Strengths

Our study is the largest study to date that evaluates the association between metformin use and mortality among breast cancer patients with diabetes. By using the Ontario databases we were able use an incident diabetic cohort which allowed us to minimize the risk of indication bias while still including over 2000 women with diabetes and breast cancer. An incident diabetic cohort minimizes indication bias since patients are at relatively similar stage of diabetes and the choice of glucose-lowering drug is most likely driven by physician preference as opposed to patient profile. Another strength of our study is that the comparator group, i.e. non-metformin, is made up predominantly of non-treated diabetic patients, as opposed to patients on other glucose lowering medications. Our study is able to identify this population since the ODD does not require drug information to categorize patients as having diabetes. Previous studies where the comparator group was patients on other glucose-lowering drugs, have been criticized since metformin users may be healthier than patients on other medications, and thus in those studies a protective effect
of metformin may be due to a healthy user bias. In general, patients who do not need
treatment with glucose-lowering medication tend to have milder diabetes that can be
controlled with lifestyle and diet alone. By comparing metformin users to predominantly
diet-controlled patients, we minimized bias that would have been due to a healthy user
effect among metformin treated patients.

5.4 Interpretation and relevance

The results are consistent with some previous studies that have not supported a
relationship between metformin use and survival following cancer, though they do contrast
other previously published studies. However, our analysis was done with careful attention
to minimize biases using methodology that has not been utilized by previous, comparable
studies. As such, our findings likely represent a better approximation of the association
between cumulative metformin use and mortality following breast cancer in our specific
population.

The implications of our findings are unclear especially in regards to the general
breast cancer population. As discussed in our limitations, our findings are generalizable to
older women who develop both diabetes and breast cancer after age 65 years and who have
a relatively short duration of diabetes. To better address a causal relationship between
metformin and survival following breast cancer, this analysis needs to be repeated in
different populations, for example women with longer standing diabetes as well as younger
women with breast cancer.

5.4.1 Consistency with other studies
Our findings contrast some previous studies that suggested that metformin has a protective effect of metformin on mortality following cancer (12-14, 67, 68, 92). However, our main analysis differed from previous work in two important ways. First, our cohort only included patients who had a new diagnosis of diabetes; whereas other studies used prevalent diabetic cohorts where mean duration of diabetes ranged from 5 to 16 years. Including cohorts with varying diabetes severity and duration may introduce indication bias since metformin tends to be used in healthier patients who have less advanced diabetes and better survival. Second, our primary analysis modeled metformin and other glucose-lowering therapies as time-varying covariates, which is more appropriate for exposures that change during a study period (93). Analyzing drug exposure from the follow-up period as being fixed at baseline introduces immortal time bias, since the time between cohort entry and date of drug prescription is ‘immortal’. By definition, a patient cannot die before receiving the prescription and thus patients who die early are less likely to be exposed to a drug (18). This bias can lead to overestimation of a drug’s effect. This was clearly illustrated in our study, whereby the strong association between ever being exposed to metformin and decreased mortality disappeared when metformin use was analyzed as a time-varying exposure as compared to as fixed exposure at baseline. Interestingly, we did not see similar changes in risk estimates between the two methodologies with other glucose lowering drugs. We hypothesize that this is due to the nature of the comparator group in the ‘never’ group for different glucose lowering drugs. For metformin, the ‘never’ metformin group was composed mostly of non drug users (77.5% - table 1) which accentuated the effect of immortal time bias since the comparator group was not exposed to a drug and thus did not have a time-dependent exposure. For sulfonylurea or insulin exposure, the ‘never’
group was more evenly made up of other drug users (i.e. metformin users), rather than patients never exposed to a drug. This minimized the effect of immortal time bias since both comparator and exposure groups were exposed to ‘immortal time’.

Our results are also inconsistent with one of the two studies that looked specifically at the effect of metformin on mortality in breast cancer patients (16, 66). Other than difference in methodology, as explained above, this inconsistency may also be explained by differences in the breast cancer population. First, Bayraktar et al. examined the effect of metformin in a population of women with triple negative breast cancer (TNBC) and found no benefit with metformin use(66). TNBC is biologically more aggressive than receptor positive disease and is often less responsive to hormonal therapy and regular breast cancer treatments which may explain it did not respond to metformin therapy(94). Another limitation to this study is that among 1448 patients included, only 130 patients had diabetes among which 63 were exposed to metformin, thus limiting their power. In contrast, He et al. looked at women with human epidermal receptor positive (HER2+) breast cancers. HER2 is a growth factor receptor that involves downstream signaling via mTOR and is over-expressed in about 25% of breast cancers(16). Knowing the HER2 status helps to guide treatment options, as these cancers tend to be more responsive to anthracycline chemotherapies as well as monoclonal antibodies that target this receptor (95). Given that the metformin may down regulate cellular proliferation via the pi3K/mTOR pathway, the authors hypothesized that metformin would be associated with an improvement in survival in this population. In multivariable analysis, metformin was associated with a 48% reduction in all-cause mortality among patients with HER2+ disease (HR 0.52, 95% CI 0.28-0.97)(16). The breast cancer subgroups studied by these two authors differed
substantially in terms of clinical features and prognosis that may explain the different association found between metformin and mortality in these two populations. We were not able to differentiate between tumour status in our study and thus we likely included various tumour subtypes in our cohort.

5.4.2 Plausibility

Both direct and indirect pathways have been proposed to explain metformin’s anti-cancer properties. Metformin is a biguanide which is believed to have an inhibitory effect on mitochondrial oxidative phosphorylation, thus inhibiting respiratory complex I (96-98). This mitochondrial inhibition leads to reduced ATP production and a state of “energetic stress” which triggers activation of AMP-activated protein kinase (AMPK) (69, 99). AMPK, a serine/threonine protein kinase, pathways are involved in regulating cellular energy metabolism, and AMPK activation leads to downstream inhibition of mammalian target of rapamycin (mTOR), a key growth factor, and subsequent reduction in protein synthesis and cellular growth (57, 58, 100, 101). mTOR signaling is often mutated and deregulated in cancer cells thus making it an important target for cancer therapies. p53, a tumour suppressor, is another important downstream target of AMPK which may be directly implicated in metformin’s anti-cancer properties (102). In vitro studies suggest that metformin p53 activation may increase cellular sensitivity to metformin mediated cell arrest (59). Metformin may also be indirectly implicated in reducing cellular proliferation independent of AMPK/mTOR pathway, through alternate signaling pathways (103).

Indirectly, metformin has documented anti-cancer effects via its insulin-and glucose-lowering properties. Metformin reduces gluconeogenesis through AMPK pathways in liver and thus increases glucose uptake in muscle cells. High levels of insulin have been
shown to be mitogenic and likely promote tumour growth by directly stimulating primitive insulin receptors (IR-A), which are present on many cells including breast cancer cells (33). By reducing insulin levels, metformin reduces ligand binding to insulin receptors and thus indirectly inhibits cellular proliferation and tumour growth by blocking growth-promoting pathways (pi3K/mTOR) that lie downstream from the insulin receptor (33). Other important receptors in the insulin receptor family that are likely down-regulated with metformin are IGF-1 as well as other “hybrid” receptors (104).

There is currently much controversy as to which of these two main described mechanisms are the most important in relaying the anti-cancer properties that are seen both in vitro and pre-clinical studies. Further research is needed to better clarify and understand this association.

5.4.3 Clinical implications

This was the largest population-based study to evaluate the effect of metformin on mortality in a cohort of women with breast cancer and diabetes. It was also the first study to attempt to minimize indication bias and to model glucose-lowering therapies as time-varying covariates. Our findings were not consistent with all studies evaluating the effect of metformin on mortality following cancer. However our study was conducted in an older population of women with a relatively short duration of diabetes, and thus our results cannot be generalized to all women with diabetes and breast cancer. Nevertheless, our findings do suggest a possible 9% reduction in breast cancer-specific mortality with long-term metformin use. More studies with longer follow-up and larger populations are needed to further explore this association. Despite these results, metformin should not be stopped in this population since it continues to be recommended as first line therapy in the
management of diabetes and the only glucose-lowering agent that has been shown to improve cardiovascular outcomes in the general diabetes population (105, 106).

5.5 Future and ongoing studies

Given the existing evidence from in-vitro and epidemiologic studies, further studies are warranted to address and clarify whether an association exists between metformin use following breast cancer diagnosis and a reduction in mortality. For example, studies among younger breast cancer patients – both pre-menopausal and younger (< age 65 years) post-menopausal women – would be useful to further shed light on the relationship between breast cancer and metformin. Another important study would be one in which the effect of metformin is evaluated in a prevalent diabetic cohort with breast cancer. If such a study is done using administrative databases, great care will need to be taken in order to minimize indication bias. Possible solutions would be matching on propensity scoring, a methodology which could account for non-random assignment of the exposure. However, in such a population a randomized controlled trial would be feasible. Different cancers have different biology and natural histories, thus further studies are needed which explore metformin’s impact on prognosis following other solid cancers, most specifically colon, lung and endometrial cancers. In addition, to further study the effect of metformin on cancer, more careful studies need to evaluate the association between metformin and cancer stage, and how this may impact mortality following cancer.

There is currently a large clinical trial (MA.32) that has randomized over 3500 patients with early stage breast cancer and no diabetes to receiving metformin or placebo(90). This study is evaluating the association of metformin with both breast cancer
recurrence and mortality. Results from this study will provide information regarding the benefit of metformin in a non-diabetic breast cancer population.

5.6 Conclusions

In summary, we conducted a large, population-based study to investigate the effects of metformin therapy on mortality following breast cancer. Cumulative exposure of metformin was not associated with either all-cause or breast cancer-specific mortality in our incident diabetic cohort when modeled as a time-varying covariate. However, our findings suggest a possible 9% reduction in breast cancer-specific mortality. We have shown that a fixed dichotomization of metformin exposure based on future exposure introduces bias and leads to misleading estimates of metformin’s effect on cancer survival. Further work is required to examine the effect of metformin in a younger population of patients with breast cancer and diabetes, as well as in a prevalent diabetic population. A better understanding of metformin’s effect on breast cancer is essential to help address the disparity in cancer outcomes between patients with and without diabetes as well as to guide diabetes treatment strategies in this population.
References


Table 1: Previous studies evaluating the effect of metformin on mortality following cancer included in our systematic review and meta-analysis.

<table>
<thead>
<tr>
<th>Source (country)</th>
<th>Cancer Site</th>
<th>Study period</th>
<th>Main outcome</th>
<th>Diabetes exposed to Metformin (%)</th>
<th>Definition of Metformin exposure</th>
<th>Results HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayraktar et al., 2011 (USA)</td>
<td>Breast</td>
<td>1995-2007</td>
<td>All-cause Mortality</td>
<td>63/130 (48)</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.82 (0.44-1.52)</td>
</tr>
<tr>
<td>He et al., 2011 (USA)</td>
<td>Breast</td>
<td>1998-2010</td>
<td>All-cause Mortality</td>
<td>NR/54</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.52 (0.28-0.97)</td>
</tr>
<tr>
<td>Garrett et al., 2012 (USA)</td>
<td>Colon</td>
<td>2004-2008</td>
<td>All-cause Mortality</td>
<td>208/424 (49.1)</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.60 (0.47-0.76)</td>
</tr>
<tr>
<td>Lee et al., 2012 (Korea)</td>
<td>Colon</td>
<td>2000-2008</td>
<td>All-cause Mortality</td>
<td>258/595 (43.3)</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.66 (0.48-0.91)</td>
</tr>
<tr>
<td>Chen et al., 2011 (Taiwan)</td>
<td>Liver</td>
<td>2003-2009</td>
<td>All-cause Mortality</td>
<td>21/53 (39.6)</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.23 (0.07-0.78)</td>
</tr>
<tr>
<td>He et al., 2011 (USA)</td>
<td>Prostate</td>
<td>1999-2008</td>
<td>All-cause Mortality</td>
<td>41/233 (17.6)</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.55 (0.32-0.95)</td>
</tr>
<tr>
<td>Romero et al., 2012 (USA)</td>
<td>Ovarian</td>
<td>1992-2010</td>
<td>All-cause Mortality</td>
<td>28/44 (63.6)</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.38 (0.16-0.90)</td>
</tr>
<tr>
<td>Sadeghi et al., 2012 (USA)</td>
<td>Pancreas</td>
<td>2000-2009</td>
<td>All-cause Mortality</td>
<td>107/302 (35.4)</td>
<td>Ever/never at cancer dx</td>
<td>HR 0.64 (0.48-0.86)</td>
</tr>
<tr>
<td>Currie et al., 2012 (UK)</td>
<td>All sites</td>
<td>1990-2009</td>
<td>All-cause Mortality</td>
<td>2843/8392 (33.8)</td>
<td>Never/every within 3 months of cancer dx</td>
<td>HR 0.71 (0.63-0.80)</td>
</tr>
</tbody>
</table>

CI, confidence interval; dx, diagnosis; HR, hazard ratio; NR, not reported.

Lega I et al. 2013, unpublished
Figure 1: Forest plot and summary HR (95% CI) of studies on the effect of metformin use on all-cause mortality in diabetic patients with cancer.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>log(Hazard Ratio)</th>
<th>SE</th>
<th>Weight</th>
<th>Hazard Ratio IV, Random, 95% CI</th>
<th>Hazard Ratio IV, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayraktar, 2011</td>
<td>-0.1988</td>
<td>0.316</td>
<td>2.2%</td>
<td>0.82 [0.44, 1.52]</td>
<td></td>
</tr>
<tr>
<td>Chen, 2011</td>
<td>-1.469675</td>
<td>0.6214</td>
<td>0.6%</td>
<td>0.23 [0.07, 0.78]</td>
<td></td>
</tr>
<tr>
<td>Currie, 2012</td>
<td>-0.34249</td>
<td>0.0609418</td>
<td>58.4%</td>
<td>0.71 [0.63, 0.80]</td>
<td></td>
</tr>
<tr>
<td>Garrett, 2012</td>
<td>-0.51</td>
<td>0.1198988</td>
<td>15.1%</td>
<td>0.60 [0.47, 0.76]</td>
<td></td>
</tr>
<tr>
<td>He (Breast), 2011</td>
<td>-0.6539</td>
<td>0.3169659</td>
<td>2.2%</td>
<td>0.52 [0.28, 0.97]</td>
<td></td>
</tr>
<tr>
<td>He (prostate), 2011</td>
<td>-0.59783</td>
<td>0.28025823</td>
<td>2.8%</td>
<td>0.55 [0.32, 0.95]</td>
<td></td>
</tr>
<tr>
<td>Lee, 2012</td>
<td>-0.4155</td>
<td>0.1659662</td>
<td>7.9%</td>
<td>0.66 [0.48, 0.91]</td>
<td></td>
</tr>
<tr>
<td>Romero, 2012</td>
<td>-0.9675</td>
<td>0.44067159</td>
<td>1.1%</td>
<td>0.38 [0.16, 0.90]</td>
<td></td>
</tr>
<tr>
<td>Sadeghi, 2012</td>
<td>-0.446</td>
<td>0.1487618</td>
<td>9.8%</td>
<td>0.64 [0.48, 0.86]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td></td>
<td>100.0%</td>
<td>0.67 [0.61, 0.73]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $\tau^2 = 0.00, \chi^2 = 7.98, df = 8 (P = 0.44); I^2 = 0$

Test for overall effect: $Z = 8.75 (P < 0.00001)$

Lega I et al. 2013, unpublished
Table 2: Glossary of epidemiological terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias</td>
<td>A systematic error, causing deviation of results or inferences from the truth.</td>
</tr>
<tr>
<td>Immortal time (time-dependent) bias</td>
<td>Bias that occurs when an exposure is classified at the start of follow-up even though subject may not actually be exposed until later in the cohort. This introduces a span of time which is ‘immortal time’ since the subject necessarily needs to remain event free until start of exposure to be classified as exposed. The introduction of immortal time bias leads to misclassification of exposure and tends to overestimate risk estimates(18, 107).</td>
</tr>
<tr>
<td>Indication (allocation) bias</td>
<td>A type of selection bias occurring in non-randomized trials where the exposure (usually a drug of interest) is given to people who are either more or less likely to suffer the outcome of interest. This bias can cause an erroneous appearance of the treatment being causally related to the outcome(108).</td>
</tr>
<tr>
<td>Healthy user bias</td>
<td>Occurs when patients who are prescribed one therapy (or exposure) are more likely to engage in healthy behaviours. If these other activities are not taken into account, the exposure will tend to overstate the effect of the exposure(109).</td>
</tr>
<tr>
<td>Protopathic bias (reverse causality)</td>
<td>Bias that is a consequence of differential misclassification of the exposure related to timing of an occurrence. It occurs when a change in exposure that takes place prior to a disease outcome is attributed as causing the outcome, when in fact it is an early manifestation of the outcome(110).</td>
</tr>
</tbody>
</table>
### Table 3: Data Sources and Validity

<table>
<thead>
<tr>
<th>Data source</th>
<th>Use</th>
<th>Dates and Validity</th>
</tr>
</thead>
</table>
| **Ontario Diabetes Database (ODD)**        | Cohort Identification         | 1997-2008  
Algorithm based identification of incident diabetics  
Compared to chart abstraction (n=3317)  
Sensitivity 0.86; Specificity 0.97 |
| **Ontario Drug Benefit Database (ODB)**    | Exposure assessment           | 1997-2010  
Days’ supply only available 1997-  
Data on patients 65 and older  
Validation of written prescription compared to ODB (n=5155), 0.7% error |
| **Canadian Institute for Health Information Discharge Abstract Data (CIHI DAD)** | Cohort identification, Risk adjustment, Outcome measurement | 1994-2010  
Agreement between CIHI DAD and hospital charts for procedures ranges between 88-95%  
Agreement in administrative data better for major versus minor procedures |
| **Ontario Health Insurance Plan (OHIP)**   | Cohort identification, Structures and processes of care | 1997-2010  
Billing claims typically provide complete capture of procedure codes  
Clinical activity from physicians remunerated via alternate funding plans (AFP) (e.g. Kingston, Ontario) are not captured |
| **Ontario Cancer Registry (OCR)**          | Cohort identification, Outcome- prostate cancer incidence and prostate cancer specific death | 1964-2010  
Estimated from both two and three source capture-recapture methods. Data completeness is estimated at 95.15% for the three source method and 95.87% for the two source method. The estimates of completeness vary by organ site and range from 91-100%  
The cause of death variable in the OCR has been validated in several studies. Validity of breast cancer-specific mortality: Sensitivity of 95% (95%CI 98.8-90.5%)  
Specificity of 88% (95% CI 92.4-79.6%) |
| **Registered Persons Database (RPDP)**     | Outcome measurement (all cause mortality), SES | 1997-2010  
Specific information regarding data accuracy is not available.  
Patient deaths are linked probabilistically to the RPDB based on the name and birth date listed on the death certificate. Patient death information from the CIHI DAD is used to corroborate/supplement RPDB data. If multiple individuals meet the linkage criteria, a patient death is not recorded in the RPDB.  
Thus, there are more people in the RPDB than are alive in Ontario. |
**Table 4:** ICD9 and ICD10 codes used to define specific baseline co-morbidities

<table>
<thead>
<tr>
<th>Disease</th>
<th>ICD-9</th>
<th>ICD-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Obstructive Lung Disease</td>
<td>491, 492, 496</td>
<td>J41, J42, J43, J44</td>
</tr>
<tr>
<td>Cardiac disease (Ischemic heart disease, heart failure)</td>
<td>410-414; 428</td>
<td>I20-25, I50</td>
</tr>
<tr>
<td>Stroke</td>
<td>430-38</td>
<td>I60-69</td>
</tr>
<tr>
<td>Hypertension</td>
<td>401-405</td>
<td>I10-13; I15</td>
</tr>
</tbody>
</table>
Table 5: Fee codes used before and after 2002 to define radiotherapy

Before 2002:

A345, C345: initial consultation for radiotherapy

A346, A348, A745, C346, C348, C745: assessment/reassessment within 6 months of initial consult

A346, A348, A745, C346, C348, C745: 2 assessment/reassessment codes within 5 weeks of first assessment/reassessment

After 2002:

<table>
<thead>
<tr>
<th>Source</th>
<th>Planning Fee Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHIP codes</td>
<td>X310-314</td>
</tr>
<tr>
<td>CCI codes</td>
<td>1YM27JADA, 1YM27JADB, 1YM27JADC, 1YM27JADE, 1YM27JADF, 1YM27JADG</td>
</tr>
</tbody>
</table>
Figure 2: Cohort design and study timeline
**Table 6**: Baseline characteristics of study population according to metformin use during the follow-up period\(^a\)

<table>
<thead>
<tr>
<th>Total (%)</th>
<th>Metformin Users (%)</th>
<th>Non-metformin-other glucose-lowering drugs (%)</th>
<th>Non-metformin-diet (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2361 (100)</td>
<td>1094 (46.3)</td>
<td>284 (12.0)</td>
<td>983 (41.6)</td>
</tr>
</tbody>
</table>

**Age**

| < 70       | 218 (9.2) | 110 (10.0) | 13 (5.6) | 95 (9.7) |
| 70-75      | 642 (27.2) | 357 (32.6) | 50 (7.8) | 235 (23.9) |
| 75-80      | 695 (29.4) | 338 (30.9) | 97 (14.0) | 260 (26.5) |
| 80-85      | 471 (20.0) | 188 (17.2) | 59 (20.8) | 224 (22.8) |
| > 85       | 335 (14.2) | 101 (9.2)  | 65 (22.9) | 160 (17.2) |

**Mean age at DM diagnosis (SD) yrs**

| 73.7 (6.0) | 72.4 (5.6) | 75.0 (6.3) | 74.8 (6.4) |

**Mean age at Bca diagnosis (SD) yrs**

| 77.4 (6.3) | 77.3 (5.6) | 79.3 (6.3) | 78.1 (6.7) |

**Mean yrs from DM to Bca dx (SD)**

| 3.7 (2.9)  | 3.9 (2.9)  | 4.3 (3.2)  | 3.4 (2.9)  |

**Mean F/u (all-cause)**

| 4.5 (3.0)  | 5.2 (3.0)  | 3.8 (2.7)  | 3.9 (2.9)  |

**Mean F/u (Bca specific)**

| 3.7 (2.8)  | 4.4 (2.8)  | 3.3 (2.5)  | 3.2 (2.7)  |

**Comorbidities**

| Cardiovascular\(^b\) | 98 (4.2)  | 37 (3.4)  | 19 (6.7)  | 42 (4.3)  |
| Hypertension        | 268 (11.4) | 115 (10.5) | 44 (15.5) | 109 (11.1) |
| COPD                | 159 (6.7)  | 48 (4.4)  | 27 (9.5)  | 84 (8.6)  |
| Stroke              | 59 (2.5)   | 20 (1.8)  | 9 (3.2)   | 30 (3.0)  |

**Glucose lowering drugs during follow-up**

| Sulfonlurea          | 818 (34.7) | 594 (54.3) | 224 (78.9) | - |
| TZD                  | 142 (6.0)  | 114 (10.4) | 28 (9.9)   | - |
| Insulin              | 263 (11.1) | 187 (17.1) | 76 (26.8)  | - |

**Mean metformin duration yrs(SD) Median**

| 2.9 (2.3) | M2.5 |

**Glucose-lowering drugs prior to breast cancer diagnosis\(^c\)**

| Metformin          | 833 (35.3) | 655 (59.9) | 94 (33.1) | 84 (8.5) |
| SU                 | 759 (32.2) | 464 (42.4) | 211 (74.3) | 84 (8.6) |
| TZD                | 48 (2.03)  | 29 (2.7)   | 15 (5.3)   | 4 (0.4)  |
| Insulin            | 97 (4.1)   | 38 (3.5)   | 50 (17.6)  | 9 (1.0)  |

**Weighted ACG score**

| Mean (SD) Median | 5.6 (12.1) | M5 | 5.0 (11.7) | M4 | 8.1 (11.4) | M8 | 5.6 (12.5) | M5 |

**Other medications (at baseline)**

| Bisphosphonates | 365 (15.5) | 155 (14.2) | 32 (8.8) | 178 (18.1) |
| Beta-blockers   | 718 (14.6) | 135 (12.3) | 46 (16.2) | 164 (16.7) |
| SSRI            | 345 (14.6) | 135 (12.3) | 46 (16.2) | 164 (16.7) |
| Statins         | 951 (40.3) | 519 (47.4) | 104 (36.6) | 328 (33.4) |

**Breast cancer treatments\(^a\)**

| Early Surgery    | 1774 (75.1) | 868 (79.3) | 187 (65.9) | 719 (73.1) |
| Chemotherapy     | 308 (13.1)  | 148 (13.5) | 30 (10.6)  | 130 (13.2) |
| Radiotherapy     | 883 (37.4)  | 445 (40.7) | 84 (29.6)  | 354 (36.1) |
| Tamoxifen        | 857 (36.3)  | 414 (37.8) | 114 (40.1) | 329 (33.5) |
| Aromatase Inhibitors | 591 (25.0) | 290 (26.5) | 66 (23.2)  | 235 (23.9) |

**Income Quintiles\(^c\)**

| 1 | 521 (22.1) | 241 (22.1) | 69 (24.4)  | 211 (21.5) |
| 2 | 534 (22.7) | 242 (22.2) | 66 (23.3)  | 226 (23.0) |
| 3 | 457 (19.4) | 210 (19.3) | 51 (18.0)  | 196 (20.0) |
| 4 | 423 (18.0) | 184 (16.9) | 43 (15.2)  | 196 (20.0) |
| 5 | 421 (17.9) | 214 (19.6) | 54 (19.1)  | 153 (15.6) |

**Rural (Y)**

| 321 (13.6) | 170 (15.5) | 35 (12.4)  | 116 (11.8) |
ACG, adjusted clinical group; Bca, breast cancer; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; SD, standard deviation; SU, sulfonylurea; TZD, thiazolidinedione; SSRI, selective serotonin receptor inhibitor.

aDrug categories based on prescriptions filled during follow-up period.
bCardiovascular disease includes hospitalizations or outpatient visits for ischemic heart disease or heart failure.
cGlucose-lowering drugs between diabetes diagnosis and breast cancer diagnosis.
dAll breast cancer treatments occurred within 1 year of breast cancer diagnosis.
eIncome quintiles are derived by linking postal codes with Canadian census data, which provide the median household income level of neighbourhood of residence.
Table 7: HR from time-varying multivariable Cox regression model for glucose-lowering therapies expressed per additional year of cumulative drug use

<table>
<thead>
<tr>
<th></th>
<th>All-cause mortality</th>
<th>Breast cancer-specific mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Univariate</td>
<td>Adjusted*</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.97 (0.93-1.07)</td>
<td>0.97 (0.92-1.02)</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>1.01 (0.97-1.05)</td>
<td>0.95 (0.89-0.99)</td>
</tr>
<tr>
<td>Insulin</td>
<td>1.14 (1.03-1.26)</td>
<td>1.01 (0.94-1.19)</td>
</tr>
<tr>
<td>TZD</td>
<td>0.90 (0.72-1.12)</td>
<td>1.06 (0.75-1.27)</td>
</tr>
</tbody>
</table>

CI, confidence interval; dx, diagnosis; HR, hazards ratio; TZD, thiazolidinedione.

*Adjusted for: cumulative sulfonylurea, insulin, TZD use, age at breast cancer diagnosis, duration of diabetes (years) prior to breast cancer, comorbidity score based on adjusted ACG score at time of cohort entry, breast cancer treatments received within 1 year of diagnosis (surgery, radiotherapy, chemotherapy, aromatase inhibitor, tamoxifen) exposure to glucose lowering drugs prior to breast cancer diagnosis (ever/never).

Table 7b: Estimates of other covariates from multivariable analyses for time-varying analysis of glucose lowering drugs.

<table>
<thead>
<tr>
<th></th>
<th>All-cause mortality</th>
<th>Breast cancer-specific mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95%) CI</td>
<td>P value</td>
</tr>
<tr>
<td>Metformin before dx*</td>
<td>1.03 (0.88-1.20)</td>
<td>0.73</td>
</tr>
<tr>
<td>Sulfonylurea before dx*</td>
<td>1.26 (1.08-1.46)</td>
<td>0.003</td>
</tr>
<tr>
<td>Insulin before dx*</td>
<td>1.12 (0.84-1.51)</td>
<td>0.43</td>
</tr>
<tr>
<td>TZD before*</td>
<td>0.99 (0.58-1.70)</td>
<td>0.98</td>
</tr>
<tr>
<td>Radiation‡</td>
<td>0.70 (0.60-0.81)</td>
<td>0.003</td>
</tr>
<tr>
<td>Surgery‡</td>
<td>0.45 (0.40-0.51)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chemotherapy‡</td>
<td>1.89 (1.56-2.28)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tamoxifen‡</td>
<td>0.76 (0.67-0.86)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aromatase Inhibitor‡</td>
<td>0.83 (0.69-1.00)</td>
<td>0.04</td>
</tr>
<tr>
<td>Year</td>
<td></td>
<td>0.54</td>
</tr>
<tr>
<td>Weighted-ACG</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age at Bca dx</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes duration</td>
<td>0.96</td>
<td>0.53</td>
</tr>
</tbody>
</table>

ACG, adjusted clinical group; Bca, breast cancer; CI, confidence interval; dx, diagnosis; HR, hazards ratio; TZD, thiazolidinedione.

*All glucose-lowering therapies defined as ever/never between diabetes diagnosis and time of breast cancer diagnosis.

‡Breast cancer treatment variables defined as ever/never occurring during first year following breast cancer diagnosis.

Bca, breast cancer; CI, confidence interval; dx, diagnosis; HR, hazards ratio; TZD, thiazolidinediones.
Table 8: Adjusted HR for never/ever time-varying drug exposure and fixed never/ever exposure from multivariable Cox regression models.

<table>
<thead>
<tr>
<th>All-cause mortality</th>
<th>Breast cancer-specific mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted HR (95% CI)*</td>
</tr>
<tr>
<td></td>
<td>Time-varying never/ever(^a)</td>
</tr>
<tr>
<td><strong>Metformin</strong>(^*)</td>
<td>0.93 (0.78-1.10)</td>
</tr>
<tr>
<td><strong>Sulfonylurea</strong>(^*)</td>
<td>1.00 (0.83-1.21)</td>
</tr>
<tr>
<td><strong>Insulin</strong>(^*)</td>
<td>2.08 (1.66-2.61)</td>
</tr>
<tr>
<td><strong>TZD</strong>(^*)</td>
<td>0.91 (0.63-1.32)</td>
</tr>
<tr>
<td><strong>Met before dx</strong></td>
<td>1.00 (0.84-1.19)</td>
</tr>
<tr>
<td><strong>SU before dx</strong></td>
<td>1.18 (0.99-1.41)</td>
</tr>
<tr>
<td><strong>Insulin before dx</strong></td>
<td>0.80 (0.57-1.09)</td>
</tr>
<tr>
<td><strong>TZD before dx</strong></td>
<td>0.64 (0.54-1.63)</td>
</tr>
<tr>
<td><strong>Surgery</strong></td>
<td>0.43 (0.38-0.50)</td>
</tr>
<tr>
<td><strong>Radiation</strong></td>
<td>0.74 (0.63-0.96)</td>
</tr>
<tr>
<td><strong>Chemotherapy</strong></td>
<td>1.90 (1.56-2.32)</td>
</tr>
<tr>
<td><strong>Tamoxifen</strong></td>
<td>0.76 (0.67-0.88)</td>
</tr>
<tr>
<td><strong>AI</strong></td>
<td>0.80 (0.66-0.96)</td>
</tr>
<tr>
<td><strong>Year</strong></td>
<td>p = 0.53</td>
</tr>
<tr>
<td><strong>Age at diagnosis</strong></td>
<td>p = &lt;0.0001</td>
</tr>
<tr>
<td><strong>Weighted ACG</strong></td>
<td>p = &lt;0.0001</td>
</tr>
<tr>
<td><strong>Diabetes duration</strong></td>
<td>p = 0.83</td>
</tr>
</tbody>
</table>

ACG, adjusted clinical group; CI, confidence interval; dx, diagnosis; HR, hazards ratio; Met, metformin; SU, sulfonylurea; TZD, thiazolidinedione.

\(^a\)Adjusted for: sulfonylurea, insulin, TZD use, age at breast cancer diagnosis, duration of diabetes (years) prior to breast cancer, comorbidity score based on adjusted ACG score at time of cohort entry, breast cancer treatments received within 1 year of diagnosis (surgery, radiotherapy, chemotherapy, aromatase inhibitor, tamoxifen) exposure to glucose lowering drugs prior to breast cancer diagnosis (ever/never).

\(^b\)Adjusted for: sulfonylurea, insulin, TZD use, age at breast cancer diagnosis, duration of diabetes (years) prior to breast cancer, comorbidity score based on adjusted ACG score at time of cohort entry and breast cancer treatments received within 1 year of diagnosis (surgery, radiotherapy, chemotherapy, aromatase inhibitor, tamoxifen).

\(^*\)HR expressed per year of drug exposure for glucose-lowering therapy after Bca diagnosis.

\(^b\)HR expressed per ever being exposed to the drug.
Table 9: Sensitivity analysis - Limiting cohort to monotherapy metformin users and patients not prescribed any glucose-lowering therapies

<table>
<thead>
<tr>
<th></th>
<th>All-cause mortality</th>
<th>Breast cancer-specific mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><em><em>HR</em>, 95% CI</em>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metformin during follow-up, per cumulative year of use</td>
<td>0.95 (0.79-1.14)</td>
<td>0.96 (0.87-1.04)</td>
</tr>
<tr>
<td>Met. before</td>
<td>1.16 (0.92-1.46)</td>
<td>1.06 (0.75-1.51)</td>
</tr>
<tr>
<td>SU before</td>
<td>1.96 (1.53-2.51)</td>
<td>2.16 (1.50-3.10)</td>
</tr>
<tr>
<td>Insulin before</td>
<td>2.51 (1.33-4.73)</td>
<td>1.48 (0.45-4.87)</td>
</tr>
<tr>
<td>TZD before</td>
<td>1.71 (0.42-6.97)</td>
<td>2.54 (0.35-18.7)</td>
</tr>
<tr>
<td>Radiation</td>
<td>0.63 (0.51-0.77)</td>
<td>0.77 (0.57-1.06)</td>
</tr>
<tr>
<td>Surgery</td>
<td>0.39 (0.32-0.46)</td>
<td>0.26 (0.20-0.35)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>1.71 (1.32-2.23)</td>
<td>2.63 (1.90-3.65)</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>0.64 (0.53-0.77)</td>
<td>0.46 (0.34-0.62)</td>
</tr>
<tr>
<td>Aromatase inhibitor</td>
<td>0.60 (0.47-0.77)</td>
<td>0.53 (0.36-0.78)</td>
</tr>
<tr>
<td>Year</td>
<td>p = 0.14</td>
<td>p = 0.78</td>
</tr>
<tr>
<td>Weighted-ACG</td>
<td>p = 0.004</td>
<td>p = 0.74</td>
</tr>
<tr>
<td>Age at Bca dx</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>DM duration</td>
<td>p = 0.62</td>
<td>p = 0.62</td>
</tr>
</tbody>
</table>

ACG, adjusted clinical group; Bca, breast cancer; CI, confidence interval; dx, diagnosis; HR, hazards ratio; Met, metformin; SU, sulfonylurea; TZD, thiazolidinedione.

*Adjusted for: age at breast cancer diagnosis, duration of diabetes (years) prior to breast cancer, comorbidity score based on adjusted ACG score at time of cohort entry, breast cancer treatments received within 1 year of diagnosis (surgery, radiotherapy, chemotherapy, aromatase inhibitor, tamoxifen) exposure to glucose lowering drugs prior to breast cancer diagnosis (ever/never).
Table 10: Sensitivity analysis – Excluding insulin users from cohort.

<table>
<thead>
<tr>
<th></th>
<th>All-cause mortality</th>
<th>Breast cancer-specific mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR, 95% CI</td>
<td></td>
</tr>
<tr>
<td>Metformin(^a)</td>
<td>0.98 (0.92-1.03)</td>
<td>0.92 (0.82-1.03)</td>
</tr>
<tr>
<td>Sulfonylurea(^a)</td>
<td>0.93 (0.88-0.98)</td>
<td>0.95 (0.85-1.06)</td>
</tr>
<tr>
<td>TZD(^a)</td>
<td>1.21 (0.93-1.54)</td>
<td>1.45 (0.99-2.12)</td>
</tr>
<tr>
<td>Met. before</td>
<td>1.09 (0.92-1.30)</td>
<td>1.03 (0.80-1.32)</td>
</tr>
<tr>
<td>SU before</td>
<td>1.47 (1.24-1.73)</td>
<td>1.27 (1.00-1.61)</td>
</tr>
<tr>
<td>Insulin before</td>
<td>1.37 (0.85-2.21)</td>
<td>0.80 (0.46-1.39)</td>
</tr>
<tr>
<td>TZD before</td>
<td>0.71 (0.38-1.34)</td>
<td>0.59 (0.22-1.54)</td>
</tr>
<tr>
<td>Radiation</td>
<td>0.67 (0.57-0.79)</td>
<td>0.81 (0.65-1.02)</td>
</tr>
<tr>
<td>Surgery</td>
<td>0.44 (0.38-0.50)</td>
<td>0.29 (0.24-0.36)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>1.85 (1.50-2.29)</td>
<td>3.15 (2.46-4.02)</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>0.75 (0.65-0.87)</td>
<td>0.71 (0.57-0.87)</td>
</tr>
<tr>
<td>Aromatase inhibitor</td>
<td>0.79 (0.65-0.96)</td>
<td>0.90 (0.68-1.19)</td>
</tr>
<tr>
<td>Year</td>
<td>p = 0.55</td>
<td>p = 0.57</td>
</tr>
<tr>
<td>Weighted-ACG</td>
<td>p &lt;0.001</td>
<td>p = 0.16</td>
</tr>
<tr>
<td>Age at Bca dx</td>
<td>p &lt;0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Diabetes duration</td>
<td>p = 0.33</td>
<td>p = 0.53</td>
</tr>
</tbody>
</table>

ACG, adjusted clinical group; CI, confidence interval; dx, diagnosis; HR, hazards ratio; Met, metformin; SU, sulfonylurea; TZD, thiazolidinedione.

*Adjusted for: Sulfonylurea, TZD use, age at breast cancer diagnosis, duration of diabetes (years) prior to breast cancer, comorbidity score based on adjusted ACG score at time of cohort entry, breast cancer treatments received within 1 year of diagnosis (surgery, radiotherapy, chemotherapy, aromatase inhibitor, tamoxifen) exposure to glucose lowering drugs prior to breast cancer diagnosis (ever/never).

\(^a\) HR expressed per cumulative year of metformin exposure.
Table 11: Sensitivity analysis – Limiting cohort to women who underwent breast surgery within the first year following diagnosis

<table>
<thead>
<tr>
<th></th>
<th>All-cause mortality</th>
<th>Breast cancer-specific mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.99 (0.93-1.06)</td>
<td>1.01 (0.86-1.20)</td>
</tr>
<tr>
<td>Sulfonylurea&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 (0.88-1.03)</td>
<td>0.92 (0.78-1.10)</td>
</tr>
<tr>
<td>Thiazolidinedione&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94 (0.72-1.23)</td>
<td>1.34 (0.79-2.29)</td>
</tr>
<tr>
<td>Insulin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06 (0.91-1.23)</td>
<td>0.77 (0.39-1.51)</td>
</tr>
<tr>
<td>Metformin before</td>
<td>0.94 (0.76-1.17)</td>
<td>0.77 (0.52-1.13)</td>
</tr>
<tr>
<td>SU before</td>
<td>1.27 (1.02-1.57)</td>
<td>1.50 (1.00-2.18)</td>
</tr>
<tr>
<td>Insulin before</td>
<td>1.23 (0.79-1.93)</td>
<td>0.79 (0.28-2.19)</td>
</tr>
<tr>
<td>TZD before</td>
<td>0.82 (0.40-1.71)</td>
<td>0.57 (0.15-2.15)</td>
</tr>
<tr>
<td>Radiation</td>
<td>0.79 (0.65-1.95)</td>
<td>0.84 (0.61-1.16)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>2.14 (1.65-2.76)</td>
<td>4.63 (3.24-6.60)</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>0.82 (0.68-0.98)</td>
<td>0.70 (0.49-0.94)</td>
</tr>
<tr>
<td>AI</td>
<td>0.94 (0.74-1.19)</td>
<td>0.82 (0.56-1.21)</td>
</tr>
<tr>
<td>Year</td>
<td>p = 0.01</td>
<td>p = 0.53</td>
</tr>
<tr>
<td>Weighted-ACG</td>
<td>p &lt; 0.0001</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>Age at Bca dx</td>
<td>p &lt; 0.0001</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Diabetes duration</td>
<td>p = 0.02</td>
<td>p = 0.14</td>
</tr>
</tbody>
</table>

ACG, adjusted clinical group; CI, confidence interval; dx, diagnosis; HR, hazards ratio; Met, metformin; SU, sulfonylurea; TZD, thiazolidinedione.

*Adjusted for: sulfonylurea, insulin, TZD use, age at breast cancer diagnosis, duration of diabetes (years) prior to breast cancer, comorbidity score based on adjusted ACG score at time of cohort entry, breast cancer treatments received within 1 year of diagnosis (radiotherapy, chemotherapy, aromatase inhibitor, tamoxifen) exposure to glucose lowering drugs prior to breast cancer diagnosis (ever/never).

<sup>a</sup> HR expressed per cumulative year of metformin exposure.