BREAST CANCER RISK AFTER METFORMIN INITIATION IN OLDER WOMEN: THE ROLE OF STUDY DESIGN, POTENTIAL CONFOUNDING BY BODY MASS INDEX, AND DIFFERENTIAL DETECTION

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology in the Gillings School of Global Public Health.

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ABSTRACT

Jin-Liern Hong: Breast Cancer Risk after Metformin Initiation in Older Women: the Role of Study Design, Potential Confounding by Body Mass Index, and Differential Detection
(Under the direction of Til Stürmer)

Several observational studies reported that metformin may reduce breast cancer risk; however, many of these studies were affected by time-related biases. Additionally, confounding by unmeasured body mass index (BMI) and differential detection for breast cancer have not been examined in metformin-breast cancer studies. The dissertation aimed to examine the relative risk of breast cancer for older women initiating metformin versus sulfonylureas, avoiding time-related bias and accounting for potential bias due to unmeasured confounding and differential screening mammography.

Using 2007-2011 US Medicare claims data, we identified cohorts of cancer-free women aged 65+ who initiated monotherapy with metformin or sulfonylureas. Hazard ratios of breast cancer were estimated comparing metformin to sulfonylureas initiators, using weighted Cox models. Unmeasured confounding by BMI and smoking was adjusted by propensity score calibration using external information from Medicare Current Beneficiary Survey 2006-2009 panels. Among new users of Medicare claims, we compared the risks of screening mammograms and screen-detected breast cancer in 12 months pre- and post-initiation between metformin and sulfonylureas initiators.

Metformin initiators did not have reduced risks of breast cancer compared with sulfonylureas initiators (Hazard Ratio: 1.08; 95% Confidence Interval: 0.81 to 1.44). Externally controlling for BMI and smoking did not affect the estimate, indicating a little independent effect of BMI and smoking on metformin relative to sulfonylureas prescribing. Metformin initiators were not only more frequently screened for breast cancer than sulfonylureas initiators, but they also had higher probabilities of screen-detected breast cancer both in 12 months before and after initiation. The results indicate possible detection bias due to differential screening mammography, but the absolute difference in screen-detected breast cancer is too small to explain observing no metformin-breast cancer association assuming a real protective effect of metformin.

This study provides no support for reduced risks of breast cancer after initiation of metformin compared with a clinical alternative, sulfonylureas, in older women. Our findings support the notion that reduced breast cancer risks in metformin users observed in previous studies is likely due to time-related biases, and emphasize the importance of conducting observational studies with rigorous, state-of-the art design to avoid observing spurious effects or missing real ones.

To my dear family.
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LIST OF ABBREVIATIONS

ACEI	Angiotensin-Converting-Enzyme Inhibitor
ADOPT	Diabetes Outcome Progression Trial
AMPK	Adenosine Monophosphate-Activated Protein Kinase-Activated Protein Kinase
AT	As-Treated Analysis
BMI	Body Mass Index
CI	Confidence Interval
CPRD	Clinical Practice Research Datalink
HR	Hazard Ratio
IQR	Interquartile Range
ITT	Intention-To-Treated Analysis
MCBS	Medicare Current Beneficiary Survey
MET	Metformin
mTOR	Mammalian Target 0f Rapamycin
OR	Odds Ratio
PS	Propensity Score
PSC	Propensity Score Calibration
PS_{EP}	Error-Prone Propensity Score
PS_{GP}	Gold-Standard Propensity Score
RD	Risk Difference
RECORD	Rosiglitazone Evaluated For Cardiovascular Outcomes and Regulation Of Glycaemia in Diabetes Trial
SMR	Standardized Morbidity Ratio
SUL	Sulfonylureas

THIN	The Health Improvement Network Database
TNBC	Triple Receptor-Negative Breast Cancer
TZD	Thiazolidinediones
WHI	Women Health Initiative

CHAPTER 1. STATEMENT OF SPECIFIC AIMS

Several observational studies have shown the beneficial effect of metformin on the risk of breast cancer [1-5]. A meta-analysis of seven observational studies found a decreased risk of breast cancer with metformin [Odds Ratio (OR): 0.83; 95% Confidence Interval (CI): 0.71-0.97)] and a slightly stronger association with longer metformin use (>3 years) (OR: 0.75; 95% CI: 0.62-0.91) [6]. However, methodological flaws exist in most studies, including the lack of well-defined active comparator group, bias from immortal-time in cohort studies, and from time-window bias in case-control studies [7]. Furthermore, little is known about unmeasured confounding by body mass index (BMI) and smoking status and whether there is differential detection for breast cancer between metformin and sulfonylureas initiators in metformin-breast cancer studies.

The goals of this study were to compare the risk for breast cancer in older adults initiating metformin with those initiating sulfonylureas, using a state of the art study design, and to explore concerns about unmeasured confounding and differential screening mammography, using data from Medicare Beneficiaries and Medicare Current Beneficiary Survey. This study does not only add to the understanding of the breast cancer risk in diabetic patients who initiate metformin or sulfonylureas, but also provides new information on some important factors at the time of treatment initiation which are usually unavailable in claims data and on differences in screening mammography.

The following specific aims were addressed in this research:

Aim 1a: To estimate the relative risk for breast cancer in metformin initiators, compared with sulfonylureas initiators, using data from Medicare Beneficiaries.

Hypothesis: We hypothesize that metformin does not affect the risk of breast cancer among older women.

Rationale: Among several observational studies indicating benefits of metformin on breast cancer, most were affected by time-related bias (i.e., immortal time bias, time window bias, and lag-time bias). These biases would lead to an apparent protective effect in the absence of a real effect or magnify any potential beneficial effect of metformin on cancer incidence [7]. Thus, the observed reduction in the risk of breast cancer associated with metformin is likely attributed to time-related bias.

Aim 1b: To examine the impact of BMI and smoking status on physicians' choice of metformin versus sulfonylureas, using data from the Medicare Current Beneficiary Survey, and to quantify unmeasured confounding by BMI and smoking status on metformin-breast cancer association.

Hypothesis: We hypothesize that BMI and smoking status affect physician decision to prescribe metformin versus sulfonylureas to diabetic patients.

Rationale: Previous studies suggest potential effects of metformin on cancer prevention; therefore, it is possible that physician may tend to prescribe metformin to those diabetic patients who are at high risk for cancer, such as current smoker and overweight/obese persons. In addition, Metformin has been shown to be moderately associated with weight loss in randomized clinical trials [8, 9], thus obese, diabetic patients are more likely to

receive metformin than a clinical alternative, sulfonylureas. Therefore, given that both factors are associated with increased risks of breast cancer in postmenopausal women [23, 25, 45], unmeasured BMI and smoking status may confound the association between metformin and breast cancer risk.

Aim 2: To compare the probability of receiving screening mammography and the incidence of screen-detected breast cancer between metformin and sulfonylureas initiators in the 12 months before and after initiation, using data from Medicare Beneficiaries

Hypothesis: We hypothesize that metformin initiators are more likely to receive screening mammography than sulfonylureas initiators in the 12 months pre- and post-initiation.

Rationale: Metformin is recommended as the first line treatment for type 2 diabetes [8], thus metformin prescribers who comply with guideline recommendations may be more likely to perform regular examinations or to recommend cancer screening tests for older patients. Previous studies also have shown that physician recommendation is one strong motivation for undergoing screening mammography [69, 70]. Therefore, women initiating metformin may be more likely to receive screening mammography and, consequently, to be diagnosed with breast cancer around the time of initiation.

CHAPTER 2. REVIEW OF LITERATURE

I. Metformin

Metformin is an oral anti-hyperglycemic agent of the biguanides class and is the first line treatment for type 2 diabetes mellitus [10]. Metformin can lower hyperglycemia by inhibiting glucose production in the liver (hepatic gluconeogenesis), decreasing the absorption of glucose in the intestine, and increasing insulin sensitivity [11]. Compared with other anti-hyperglycemic drugs, metformin has low risks of hypoglycemia and minor side effects. The common side effects of metformin include stomach or abdominal discomfort, diarrhea, muscle pain or weakness, and decreased appetite. Additionally, metformin may induce lactic acidosis, which is a rare but severe adverse effect and usually occurs in diabetic patients with impaired renal function [12].

Metformin has been shown to help facilitate weight loss [8, 9] and to effectively delay or prevent developing diabetes in patients with pre-diabetes [8, 9, 13, 14], identified by impaired fasting glucose, impaired glucose tolerance, or elevated HbA1c according to American Diabetes Association [10]. In addition to treatment of hyperglycemia, metformin is also used to treat polycystic ovary syndrome [15, 16]. Some studies also suggest that metformin may have beneficial effect on prevent the cardiovascular disease [17-19].

Recently, metformin has received much attention due to its potential beneficial effect on cancer prevention and treatment, in particular, on breast cancer [20-22]. Evidence from

preclinical and clinical studies has suggested that metformin has anti-tumor ability and may reduce incidence and mortality of breast cancer [23, 24].

II. Plausible mechanism for antitumor action of metformin

The mechanism of metformin action on breast cancer is unclear but it is generally believed to involve both direct and indirect action through mediating adenosine monophosphate-activated protein kinase-activated protein kinase (AMPK). AMPK is an enzyme which plays an important role in cellular energy homeostasis. Metformin can activate AMPK to directly inhibit cellular protein synthesis and cell proliferations in both normal and cancer cells, through suppression of the mammalian target of rapamycin (mTOR) pathway. mTOR is a type of protein which regulates protein synthesis and cell growth/proliferation. On the other hand, activation of AMPK also can inhibit transcription of key gluconeogenesis genes in the liver and stimulate glucose uptake in muscle; as a result, metformin can reduce the levels of circulating glucose and insulin and can increase the insulin sensitivity, thus indirectly inhibiting carcinogenesis and cancer prognosis [25, 26].

III. Clinical Studies of the effect of metformin on the risk of breast cancer

The risk of breast cancer associated with metformin has been examined in ten observational studies and two clinical trials [1-5, 27-32]. Four studies compared the breast cancer risk of metformin with no use of metformin, three of which reported that metformin reduced the risk of breast cancer [1, 2, 4, 28]. The strongest effect was observed in a case-control study within UK Clinical Practice Research Datalink (CPRD, formally known as General Practice Research Database, GPRD) [1]. Among women aged 30-79 and diagnosed with type 2 diabetes, long-term use of metformin (≥ 40 prescriptions) showed a strong effect on preventing breast

cancer, compared with no use of metformin [Odds Ratio (OR): 0.42; 95% Confidence Interval (CI): 0.21-0.87]. A similar but weaker beneficial effect was observed in one Danish case-control study and one UK cohort study [2, 4]. The case-control study nested in women with type 2 diabetes in Danish Medical Registries found that metformin use was associated with a reduced risk of breast cancer (OR: 0.81; 95% CI: 0.63-0.96) [2]. In a cohort study of the Diabetes Audit and Research in Tayside Study, Scotland, metformin user had a decreased risk of breast cancer compared with nonusers of metformin matched on the year of diabetes diagnosis [Hazard Ratio (HR): 0.60; 95% CI: 0.32-1.10] [4]. However, among older women with diabetes and receiving treatment of glargine or nonglargine insulin enrolled in Medicare, metformin was shown to increase the risk of breast cancer compared with no use of metformin (HR: 1.28; 95% CI: 1.05-1.57) [28].

The breast cancer risks of metformin and other oral anti-diabetic drugs have been compared with one another in six studies [5, 27, 29-31]. The significantly inverse association between metformin and breast cancer was only found in a cohort study of PHARMO Record Linkage System in the Netherlands [5]. The risk of breast cancer slightly decreased in new users of metformin, compared with new users of sulfonylureas (HR: 0.95; 95% CI: 0.91-0.98). The other study comparing metformin use versus sulfonylureas use in the CPRD showed no evidence of a reduced risk of breast cancer (HR: 1.04; 95% CI: 0.79-1.37) [30]. Two UK cohort studies comparing sulfonylureas with metformin also showed no altered risk of breast cancer, using data from THIN (HR: 1.04; 95% CI: 0.79-1.37) and CPRD (HR: 0.98; 95% CI: 0.61-1.41) [27, 29]. No beneficial effect of metformin on breast cancer was also found in re-analyses of two clinical trials: A Diabetes Outcome Progression Trial (ADOPT) and Rosiglitazone Evaluated for Cardiovascular Outcomes and Regulation of Glycaemia in Diabetes (RECORD) [31]. In ADOPT

with a median treatment and follow-up time of 4.0 years, six (1.0%) and six (0.9%) patients developed breast cancers among 590 patients randomized to metformin compared with 645 patients randomized to rosiglitazone, with a HR of 1.09 (95% CI: 0.35-3.41). In the RECORD study patients on sulfonylureas were randomized to metformin or Rosiglitazone and treated and followed over 5.5-years. Of the 554 patients randomized to metformin, 8 (1.4%) developed breast cancer compared with 4 (0.7%) among 562 patients randomized to rosiglitazone, resulting in a HR of 2.04 (95% CI: 0.61-6.83).

Additionally, one study examined the risk of breast cancer associated with metformin use among postmenopausal women from Women Health Initiative (WHI) clinical trials with an average of 11.8 years follow-up [3]. Diabetic women initiating metformin had a decreased risk of breast cancer compared with non-diabetic women (HR: 0.75; 95% CI: 0.57-0.99). The breast cancer risk in metformin users has been also examined over time [32]. Metformin users showed a pattern of decreasing breast cancer over time. The incidence rate ratio was 0.73 (95% CI: 0.56-0.96) during months 6-24, 0.91 (95% CI: 0.70-1.17) during months 25-60, and 0.82 (95% CI: 0.61-1.10) during months 60+, compared with the initial 6 months after starting treatment.

Although the results are not consistent, a recent meta-analysis found a decreased risk of breast cancer associated with metformin (OR: 0.83; 95% CI: 0.71-0.97) and a stronger association with longer metformin use (>3 years) (OR: 0.75; 95% CI: 0.62-0.91) [6]. This beneficial effect was subsequently supported by another meta-analysis (OR: 0.94; 95% CI: 0.91-0.99) [33]. However, results and study designs of these published studies are very heterogeneous and thus the usefulness to combine these into a single summary estimate is questionable. Choice of comparison group, for example, varied widely across studies. Estimates comparing metformin

with no use of metformin suggest more beneficial effect than those comparing metformin with other anti-diabetics drugs.

IV. Methodological concerns on available literature

Despite the vast body of observational studies on the breast cancer risk of metformin, majority of these studies had flaws in study design (Table 2.1). The most important problem are time-related biases, as pointed out by Suissa and Azoulay [7]. Time-related biases, including immortal time bias, time-window bias, and bias from time lag and latency, likely exaggerate the potential beneficial effect of metformin on cancer incidence. Thus, the reduced risk of breast cancer observed in current literature could be attributed, at least in part, to time-related biases. Among the 12 metformin-breast cancer studies, five are subject to time-related biases: two suffer from immortal time bias [27, 30]; two from time-window bias [1, 2]; and two from time-lag bias [4, 27]. In addition to time-related biases, numerous methodological issues and limitations should be also noted. Firstly, some studies used Cox models with time-varying exposure but failed to consider time-varying confounders [3, 30]. Any prescription change of oral anti-diabetic medications highly correlates with control of blood glucose. Patients with poorly controlled glucose level are more likely to switch to or to receive additional anti-diabetic drugs. Secondly, the WHI study may be subject to recall bias and exposure misclassification because information on diabetes diagnosis and medication was retrieved from questionnaires in year 0, 1, 3, and 6 [3]. Third, in re-analyses of ADOPT and RECORD clinical trials, the number of breast cancer cases was less than ten, which may not be large enough to generate a valid estimate with a precise confidence interval [31]. Also, clinical trial tends to recruit young and healthy patients, and thus, the results may not be generalizable to the overall population of metformin users. For the only study which examined the breast cancer risk of metformin over time, it is misleading to imply

beneficial effects of long-term metformin use by comparing metformin user who had longer follow-up time with metformin users who had follow-up time less than 6 months [32]. Characteristics between patients with long follow-up time and those with short follow-up time might be different. For example, healthier patients are likely to have longer follow-up times and maybe less likely develop cancer. Lastly, confounding is not well controlled for in some studies, even in the studies with active comparison groups [5, 29]. Although both metformin and sulfonylureas are used for diabetes treatment, patient characteristics for these two drugs may differ, such as comorbidity and concurrent medication use.

Apart from the methodological flaws and limitation described above, unmeasured confounding by body mass index (BMI) and smoking status and detection bias are another problem which has not been addressed in metformin-cancer studies. BMI and smoking are positively associated with the risk of several types of cancers. For breast cancer, obese women after menopause have about 20% higher risk of developing breast cancer than women with normal weight [34], but it remains controversial whether smoking affects breast cancer incidence [35-37]. Given the beneficial effect of metformin on weight loss and a heated discussion about benefits of metformin on cancer, physicians may preferably prescribe metformin to overweight or obese patients, as well as smokers [10]. Thus, the association between metformin and breast cancer could be confounded by BMI and smoking status. Some studies controlled for BMI and smoking status. The potential magnitude of confounding by BMI and smoking has not been evaluated in any study, however.

Detection bias has been studied in diabetes-cancer studies, but little is known in metformin-cancer studies [38]. It is reasonable to suspect the existence of detection bias in metformin-cancer studies of older population. According to the guidelines, metformin is

recommended as the first line treatment for diabetes, except for patients with chronic kidney disease [10]. Therefore, physicians who prescribe metformin as initial treatment rather than any other anti-diabetic drug follow the guidelines and might also be more likely to perform regular examinations or recommended cancer screening tests for older patients who are at high risk for several types of cancers compared with their peers who do not. Differential detection before initiation could reduce cancer incidence by early diagnosis and removal of (asymptomatic or pre-clinical) cancer. On the other hand, differential detection after initiation may lead to an increased risk immediately following treatment initiation. This speculation could be supported in breast cancer by the UK study [32]. Among metformin users aged 40 or over in the CPRD, the breast cancer risk is higher in the first 6 months than later. Unfortunately, no study has attempted to address this issue.

Due to concerns about the important time-related bias and potential confounding in metformin-breast cancer studies, the effect of metformin on the risk of breast cancer remains unresolved [7]. Thus, we conducted a cohort study to investigate the effect of metformin on the risk of breast cancer in postmenopausal women using a state of the art nonexperimental study design, and to assess the impact from unmeasured confounding by BMI and smoking status as well as detection bias, using data from a US wide sample of Medicare beneficiaries.

V. Significance

Breast cancer is the most common cancer for women worldwide and in the United States. The Surveillance, Epidemiology, and End Results (SEER) program estimated approximately 232,340 new cases of breast cancer in the year of 2013, accounting for 14.1% of all new cancer cases in the US [47]. The cost of breast cancer care in 2010 was estimated at 16.5 billion dollars

in US, the highest among all cancer sites [48]. Diabetes is positively associated with the risk of breast cancer, increasing risk around 20 to 40% in women [49]. As a preferred treatment for diabetes, metformin's potential benefits on breast cancer risk may have clinical interest and implications. In addition, the National Cancer Institute highlighted the potential effects of metformin on cancer prevention as well as some important ongoing clinical trials in their April 2013 Cancer Research Update [50]. Therefore, it is needed to elucidate whether metformin reduces the risk of breast cancer in high risk populations, by conducting an observational study with state of the art methodology and rigorous design, which avoids immortal time bias and addresses potential confounding by BMI and differential detection for breast cancer.

VI. Tables

TABLE 2.1. Methodological concerns in previous metformin-cancer studies

Study	Study Design	Exposure vs Comparison	Methodological Concerns or Limitations
Currie 2009 [27]	Cohort	MET vs SUL	Immortal Time Bias; Time-Lag Bias
Libby 2009 [4]	Cohort	MET vs No MET	Time-Lag Bias
Home 2010 [31]	RCT	MET vs ROSI MET vs Glyburide	Re-analysis of clinical randomized trial (ADOPT); very small number of breast cancer cases (<10 each group)
Home 2010 [31]	RCT	MET+SUL vs MET+ROSI	Re-analysis of clinical randomized trial (RECORD); very small number of breast cancer cases (<10 each group)
Bosco 2011 [2]	Case-Control	MET vs No MET	Time-Window Bias
Morden 2011 [28]	Cohort	MET vs No MET	Prevalent users; Examining metformin effect on a background of glargine or non-glargine insulin
Bodmer 2012 [1]	Case-Control	MET vs No MET	Time-Window Bias
Chlebowski 2012 [3]	Cohort	DM DX+MET vs NO DM	Recall Bias from Survey; Exposure misclassification; Failure to consider time-varying confounders
Redaniel 2012 [30]	Cohort	SUL vs MET	Immortal Time Bias; Failure to consider time-varying confounders
Ruiter 2012 [5]	Cohort	MET vs SUL	Not considering other potential confounders, such as comorbidity and current medication use.
Van Staa 2012 [32]	Cohort	Duration of MET use	It is misleading to imply beneficial effect of long-term metformin use by comparing metformin user who had longer follow-up time with metformin users who had follow-up time less than 6 months.
Qui 2013 [29]	Cohort	SUL vs MET	Not considering other potential confounders, such as comorbidity and current medication use.

Abbreviations: DM: Diabetes; MET: metformin; SUL: sulfonylureas; No MET: no use of metformin; ROSI: rosiglitazone; RCT: Randomized Trial

CHAPTER 3. METHODS

I. Overall Strategy

This study conducted a cohort study with new user design to examine the risk of breast cancer after metformin compared with sulfonylureas initiation, using 2007-2011Medicare claims data. Cross-sectional studies was also conducted to quantify the effects of BMI and smoking status, which are unmeasured in Medicare claims data, on initiation of metformin versus sulfonylureas as external control, using data from the Medicare Current Beneficiary Survey (MCBS) 2006-2009 panels. We further assessed the impacts of unmeasured confounding by BMI and smoking status on the association between breast cancer risk and metformin in Medicare data. In addition, to investigate the potential detection bias in a study of the effects of anti-hyperglycemic drugs on breast cancer risk, we described patterns of breast cancer screening examinations over 12-months period pre- and post-initiation of metformin and sulfonylureas by summarizing frequency of screening mammography within 3-month intervals in the new user cohorts based on Medicare claims data, and estimated the risk difference of receiving screening mammography comparing metformin initiators with sulfonylureas initiators over a total of two-year period.

II. Data Source

1. Medicare Beneficiaries

Medicare is funded by United States (US) federal government and is administered by the Center of Medicare and Medicaid Services. Medicare provides medical coverage for citizens aged 65 years and older, with certain disabilities, or with End-Stage Renal Disease (ESRD). In 2010, 47 million persons were enrolled in Medicare, of whom 8 million were disabled persons or persons with ESRD under age 65 [40]. The Medicare database is composed of three types of data files: Part A (hospital claims), Part B (outpatient physician services), and Part D (dispensed prescription claims), capturing patient information including demographics, diagnosis and procedure codes, along with claims for dispensed prescriptions. All clinical data are recorded by the Classification of Disease 9th Revision (ICD-9), Current Procedural Terminology-4 (CPT-4) codes, and the Healthcare Common Procedure Coding System (HCPCS). Dispensed prescriptions are coded using National Drug Codes (NDCs). In this study, we identified our study cohort from twenty percent of the entire fee-for-service Medicare population.

2. Medicare Current Beneficiary Survey (MCBS)

The Medicare Current Beneficiary Survey (MCBS) is a continuous, multipurpose survey of a nationally representative sample of the Medicare beneficiaries, conducted by the Office of Information Products and Data Analysis (OIPDA) of the Centers for Medicare & Medicaid Services. The MCBS contains data on socioeconomic, demographic characteristics, health status and functioning, health care use, health insurance coverage, and Medicare claims for survey participants. This survey has a high response rate (between 85% and 95%) and very high degree

of data completeness [41-43]. The MCBS includes two modules: Access to Care, and Cost and Use Files, and we used the module of Cost &Use Files in this study.

III. Study Population

1. Study Cohort from Medicare Beneficiaries

Study cohort for Aim 1a (Breast Cancer Risk)

Eligibility requirements for women to be included in this study were: (1) continuous enrollment in Medicare Part A, B, and D for at least 6 months prior to treatment initiation during the period from 2007 to 2011, (2) age of 65 years or more at treatment initiation, (3) new users of metformin or sulfonylureas with at least 1 refill within 90 days after end supply of the index prescription, and (3) free of kidney disease and any type of cancers except for non-melanoma skin cancer within 6 months prior to treatment initiation. New use was defined as not receiving any anti-diabetic treatment within 6 months prior to initiation of metformin or sulfonylureas, including oral and injection anti-diabetic drugs and insulin or its analogues. Patients were categorized into the cohorts of metformin or sulfonylureas according to the first prescription (index prescription). To increase the likelihood that patients are actually exposed to the drug, eligible new users were required to have at least one refill of index prescription within 90 days after end of drug supply of index prescription. The date of the first refill was defined as the index date.

In this study, we did not restrict the study cohort by requiring diagnosis of diabetes prior to treatment initiation because both metformin and sulfonylureas are used almost exclusively for treatment of type 2 diabetes. Although women with polycystic ovary syndrome (PCOS) may receive metformin for treatment, PCOS occurs in older women very

rarely. Thus, given our study cohort consisting of women aged 65 and over, all initiators of metformin or sulfonylureas can be considered as truly having type 2 diabetes mellitus.

Study cohort for Aim 2 (Screening Mammography)

To address different effects due to differential detection pre- and post-initiation, we included two study cohorts in the study of Aim 2. One was the new user cohort which was used to examine use of screening mammography over a two-year window of 12 months pre- and post-initiation, primarily focusing on the period of 12 months prior to initiation. The other study cohort was the cancer-free cohort mimicking a cohort study on breast cancer incidence, which only examined use of screening mammography in 12 months after initiation.

Similar inclusion criteria of Aim 1a (Breast Cancer Risk) were used to select study cohorts for Aim 2. For the new user cohort, we identified women aged 65 or older who initiated monotherapy of metformin or sulfonylureas between 2008 and 2010, in order to give potential two-year enrollment period before and after initiation for examining receipt of screening mammography. Initiation was defined as having at least one refill within 90 days after end of drug supply of the initial prescription and having at least 12 months of continuous Part D enrollment prior to initiation without use of any anti-diabetic drugs. Patients were classified as new users of metformin or sulfonylureas according to the initial prescription and the date of the first prescription was defined as the index date (i.e., initiation). Eligible patients were also required to have enrolled in Part A and B continuously for $\geq$ 24 months pre-initiation and $\geq$ 12 months post-initiation, thus patients who died or disenrolled Medicare Part A and B within 12 months after initiation were excluded from the study.

A study examining cancer incidence commonly requires a cancer-free study population. Thus, we had the cancer-free cohort to correctly assess receipt of screening mammography within 12 months following treatment initiation. This cancer-free cohort was a subgroup of the new user cohort, which included eligible initiators without a diagnosis of any cancer except for non-melanoma skin cancer within 12 months prior to initiation.

2. Study Cohort from Medicare Current Beneficiary Survey (MCBS)

To address the specific aim 1b (Unmeasured Variables), we conducted an external validation study using data from MCBS 2006-2009 panels. We identified new users of metformin or sulfonylureas of both genders from all MCBS participants, using data from the file of Prescribed Medicine Events in MCBS Cost & Use files (equivalent to Medicare Part D prescription files). The number of eligible patients in MCBS was expected small, thus initiation was defined by requiring only one prescription and new use was defined as no prescription for metformin or sulfonylureas in 6 month prior to initiation of monotherapy of metformin or sulfonylureas.

IV. Variables of Interests

1. Variables of Interests for Specific Aim 1a (Breast Cancer Risk)

Exposure and Follow-up

Exposure was defined as metformin or sulfonylureas according to dispensed prescription and its refill claims in Medicare Part D.

Two approaches were undertaken to define follow-up time: the as-treated (AT) and the intention-to-treat (ITT) analyses. In addition, because the primary outcome was breast

cancer which has a long preclinical phase, we incorporated the assumptions of induction and latency periods of 180 days each in the analysis. In AT approach, follow-up started on 180 days after the index date, and ended with the earliest of the following events: 180 days after augmentation or discontinuation of treatment, any cancer diagnosis except for non-melanoma skin cancer, death, end of enrollment in Medicare part D for greater than 1 month, or end of study (December 31st, 2011). Augmentation was defined as a subsequent addition of other anti-diabetic drugs to index prescription. Treatment discontinuation was defined as no further refill within the days of supply plus a 90-day grace period. Secondly, ITT analysis would follow-up patients from 180 days after the index date and until the date of any cancer diagnosis except for non-melanoma skin cancer, death, or end of study, irrespective of any treatment discontinuation or treatment change.

Outcome

The outcome was diagnosis of incident breast cancer during follow-up, including both *in situ* and invasive breast cancer. The definition for cancer event was at least two diagnoses of breast cancer on different dates within 60 days. The date of the first diagnosis was defined as outcome date. This algorithm has been validated in the Medicare data [44].

2. Variables of Interests for Specific Aim 1b (Unmeasured Variables)

Exposure

The main exposures of interest were BMI and self-reported smoking status. Data on height, weight, and smoking status were extracted from MCBS Cost & Use Survey data. BMI was calculated by weight (kilogram) divided by height (meter) squared, and was treated as a continuous variable and a categorical variable as follows: (1) <25 as normal; (2) ≥25 and

<30 as overweight; (3) ≥30 as obese, according to WHO criteria [45]. Smoking status was considered as a binary variable (never and ever smoker). Missing data on weight, height, and smoking status are possible in MCBS data, but was uncommon (< 5%).

Outcome

In the validation study of MCBS, the outcome was initiation of metformin, compared with initiation of sulfonylureas. Definition has been described above.

3. Variables of Interests for Specific Aim 2 (Screening Mammography)

Screening Mammography

We used the following the Healthcare Common Procedure Coding System (HCPCS) codes to select all mammograms for the study cohort: G0202-G0205, 76091-76092, 77051-77052, and 77056-77057. Mammograms were further classified as screening versus diagnostic test based on a claims-based algorithm [67]. Briefly, mammograms were considered as screening test if they were coded as screening mammography without a previous mammogram within prior 9 months and without any breast cancer diagnosis in the prior year. This algorithm has been validated in Medicare claims with a high positive predictive value (PPV) of 94.9% [67].

Screen-Detected Breast Cancer

After distinguishing screening from diagnostic mammograms, we also used the Fenton algorithm to identify incident screen-detected breast cancers [68]. This algorithm has a high PPV of 88.0% among Medicare enrollees [68]. The Fenton algorithm classifies screening mammograms as positive to detect breast cancer by requiring a breast cancer

diagnosis within 123 days post-mammogram and a breast-directed surgery within a year following the diagnosis, or by a diagnosis of carcinoma in-situ within 286 days post-mammogram with a subsequent mammogram within 82 days following the diagnosis. The detail of this algorithm and the results were shown in Supplemental Figures S4-S5. To evaluate the performance of screening mammography in our cohorts, we calculated the screening detection rate for breast cancer by dividing the number of screen-detected breast cancers by the number of screening mammograms.

Incident Breast Cancer

In the cancer-free cohort, any incident breast cancer during 12-month follow-up was another outcome of interest, irrespective of whether it was detected by screening or due to symptoms. To be similar to the Fenton algorithm, we required a breast-directed surgery within a year following a breast cancer diagnosis code, including invasive and carcinoma in situ, to ascertain the breast cancer case.

V. Confounding Control

Relevant to Specific Aims 1a (Breast Cancer Risk) and 1b (Unmeasured Variables) of the proposed study, all covariates were defined based on available information within the 6-month period prior to and on the index date. Covariates of interest include:

(1) Demographics: Age in years (continuous variable), Gender (female or male), Race (White, Black, Others).

(2) Co-morbidity: Benign Breast Disease, Benign Neoplasma of Breast, Chronic Obstructive Pulmonary Disease (COPD), Chronic Heart Failure (CHF), Chronic

Kidney Disease (CKD), Acute Kidney Injury (AKI), Ischemic Heart Disease, Hypertension, Osteoporosis. All co-morbidity variables were categorized into binary variables (Yes or No), defined as at least one diagnosis code.

(3) Medications: Estrogen, Progestin, Statins, Bisphosphonates, ACE inhibitors, ARBs, Beta Blockers, Antidepressant, Digoxin, Oral Contraceptives, Calcium Channel Blockers, Cholesterol Absorption Inhibitor, Loop Diuretics, non-Loop Diuretics. All medication variables were categorized into binary variables (Yes or No), defined as at least one prescription or refill records.

(4) Healthcare System Use: Number of Hospitalization, Days of Hospitalization, Number of Physician Visit, Number of Emergency Room Visit, Number of Mammography Exams, Number of Lipid Tests. All healthcare system use variable were treated as both continuous variables and categorical variables.

We applied propensity score (PS) methods to control potential confounding [46]. For each patient, the probability of receiving metformin versus sulfonylureas was estimated by using a logistic regression model which included all covariates as independent variables, as known as estimated propensity score (EPS). We implemented the standardized mortality or morbidity ratio (SMR) weighting method to balance two cohorts on covariates at baseline. In SMR weighting method, we controlled for all these covariates by standardizing to their distribution in the metformin initiators using weights of 1 for metformin initiators and the odds of propensity score for sulfonylureas initiators [47].

To address the specific Aim 2 (Screening Mammography), we assessed the confounding effect by age, gender, race/ethnicity, number of physician visits, and calendar year of initiation.

VI. Statistical Analysis

All analyses were performed with the SAS software, 9.3 version.

1. Analyses to address Specific Aim 1a (Breast Cancer Risk)

We began with descriptive analyses of study cohorts by variables listed in confounding control, and repeated with adjustment of SMR weighting to examine the balance between two cohorts after controlling confounders.

The relative risk of breast cancer comparing metformin initiators to sulfonylureas initiators was initially examined with AT approach as the primary analyses, and then repeated with ITT approach as the secondary analyses. For each cohort, we calculated incidence rates of breast cancer per 100,000 person-years and use Kaplan-Meier methods to plot cumulative incidence of breast cancer. A Cox proportional regression models was used to estimate the crude and adjusted hazard ratios (HRs) of breast cancer and their corresponding 95% confidence intervals (CIs). The proportional hazard assumption was assessed by an addition of an interaction term between cohort and log survival time and by plotting –ln(ln(estimated survivor function)) as a function of time on the logarithmic scale. To explore the trend of hazard ratio over time, we further estimated hazard ratios for sequential 6-month intervals since treatment initiation.

We also performed subgroup analyses for age, race/ethnicity and use of statin at initiation. It has been shown that subtype of breast cancer varies by age and race [48, 49]. African Americans are more likely to develop triple receptor-negative breast cancer than white women. Thus, age and race/ethnicity were likely to be effect modifiers on the association between metformin and breast cancer incidence. Statin, HMG-CoA reductase inhibitors, has been linked to reduce cancer incidence [50, 51]. Although clinical evidence suggests that statins does not

affect the incidence of breast cancer, a recent observational study of a large national cohort showed that statins may significantly improve cancer mortality after diagnosis of breast cancer [52]. Given the possible chemopreventive effect from statin on breast cancer, we evaluated the risk of breast cancer associated with metformin in strata based on presence or absence of statin prescription at baseline (i.e., defined in 6 months prior to treatment initiation).

Several sensitivity analyses were pre-planned.

(1) To evaluate the robustness of the assumptions of induction and latency periods made in the study, the main analysis was repeated with various lengths of time for the induction period ranging from 0 to 365 days and for the latency period ranging from 0 to 730 days.

(2) Six-month washout period may not be long enough to successfully capture new users and to accurately assess covariates at baseline. Thus, we repeated the study in which increases the washout period for new use and covariate assessment to 12 months.

(3) Carcinoma in situ is an early form of cancer and is highly possible to transform into invasive cancer but not necessarily. Given greater clinical interests in invasive carcinoma than carcinoma in situ, we further restricted the outcome of interest to invasive breast cancer only.

(4) To minimize the potential misclassification in defining treatment use during follow-up and diabetic patients, we repeated the main study with a longer grace period of 180 days and restricting to new users who had a diagnosis code for diabetes within 6 months before initiation, respectively.

(5) Due to under-coding of renal disease, we conducted the analyses including prior renal disease and excluding only those patients with severe renal disease (i.e., chronic kidney disease stage 4 and 5).

(6) Given the unresolved concerns that sulfonylureas may increase the risk of breast cancer, we also conducted sensitivity analyses of using two comparator groups: (1) new users of Thiazolidinediones (TZDs) or Incretins; (2) diabetic patients who started treatment of angiotensin-converting-enzyme inhibitors (ACE inhibitors) and did not receive any prescription of anti-diabetic drugs. ACE inhibitors were chosen because of the following reasons. First, the older persons have a high prevalence of hypertension. Secondly, ACE inhibitors are considered as one of the first line treatment in hypertension. Patients starting treatments with ACE inhibitors are less likely to have severe hypertension, thus patient characteristics may not be heavily weighted in cardiovascular diseases, compared with metformin initiators. Lastly, hypertension is not a risk factor for breast cancer and no evidence shows that ACE inhibitors are associated with an altered risk of breast cancer.

2. Analyses to address Specific Aim 1b (Unmeasured Variables)

In this cross-sectional study, we calculated the prevalence of variables of interest by study cohort, and used a logistic regression model to estimate odds ratio (OR) of receiving metformin versus sulfonylureas and their corresponding 95% confidence intervals. Analysis was adjusted in a multivariable model, as described in the section of Confounding Control.

The results from this validation study were used to calculate the extent of potential confounding by BMI and smoking status for our main analysis (Aim 1a: Breast Cancer Risk) and

to further correct the effect estimates in the main study using propensity score calibration (PSC).[53] In line with our main study (female cohort), we use data from women only in the validation study. We quantified these associations independent of other covariates, fitting propensity score model equivalent to the one in the main study as far as possible (the number of initiators may limit the number of covariates that can be included in PS models in the validation study).

To implement PSC, two PSs were estimated within the MCBS data: (1) the error-prone PS ($PS_{EP,\ MCBS}$) based on the same variables used in the main study, and (2) the gold-standard PS ($PS_{GS,\ MCBS}$) based on not only the same variables in the main study but also additional unmeasured variables (i.e., BMI and smoking status). We fit a model for the simple linear regression of $PS_{GS,\ MCBS}$ on $PS_{EP,\ MCBS}$ and treatment (Equation 3.1). Then, based on the estimated parameters from this model, we imputed a new PS (considered as "Gold-standard", $PS_{GS,\ main}$) with the original PS (considered as "error-prone", $PS_{EP,\ main}$) in the main study. The association of metformin-breast cancer was estimated in a Cox model, adjusted by SMR weighting.

$$PS_{GS} = \beta_0 + \beta_1(PS_{EP}) + \beta_2(Treatment) \qquad \text{(Equation 3.1)}$$

3. <u>Analyses to address Specific Aim 2 (Screening Mammography)</u>

In the new user cohort, we began with a description of metformin and sulfonylureas initiators by receipt of screening mammography during a two-year window of 12 months pre- and post-initiation. The day of initiation was indexed as Month 0, and was included in the month following initiation (Month 1). Frequency of initiators receiving a screening mammogram was summarized within sequential 3-month and 12-month intervals from Month -12 (before initiation)

to Month 12 (after initiation), respectively. Plots of frequency by time interval were used to visually compare the temporal trends of each event by treatment. Incident breast cancer detected at screening was calculated over 12-monthly intervals, given expected small numbers. We estimated risks and risk differences (RDs) and their 95% CIs of each event during each time interval comparing metformin to sulfonylureas initiators.

In the cancer-free study, we repeated all analyses during the time window of 12 months after initiation only and calculated RD of total breast cancer incidence comparing metformin to sulfonylureas groups in 12 months after initiation. Given the fact that screening mammography prior to initiation is likely associated with receiving subsequent screening tests after initiation, we additionally included the variable of prior screening in the propensity score model for confounding control. Furthermore, the analyses in this cancer-free cohort were stratified by receipt of screening mammography in 12 months prior to initiation.

CHAPTER 4. BREAST CANCER RISK IN OLDER WOMEN INITIATING METFORMIN VERSUS SULFONYLUREAS

I. Introduction

Breast cancer is the most common cancer and is the second leading cause of cancer death for women in the United States . The cost of breast cancer care in 2010 was estimated at 16.5 billion dollars in US, the highest among all cancer sites [55]. Diabetes increases breast cancer risk by 20-40% in women [56]. As the first-line treatment for type 2 diabetes [10], metformin has received much attention due to its potential beneficial effect on cancer incidence and outcomes, in particular, on breast cancer [20-22].

Evidence from preclinical and clinical studies suggests that metformin has anti-tumor properties and may reduce incidence and mortality of breast cancer [23, 24]. A meta-analysis of seven observational studies found a 17% decreased risk of breast cancer associated with metformin, and reported that metformin use for 3 years or longer reduced the risk of breast cancer by 25% [6]. Despite several observational studies indicating chemopreventive effects of metformin on breast cancer, concerns have been raised that many of these studies were subject to time-related biases (e.g., immortal time bias and time-window bias) which would lead to an apparent protective effect in the absence of a real effect or magnify any potential beneficial effect of metformin on cancer incidence [7].

Body mass index (BMI) and smoking may confound the metformin-breast cancer association, but were rarely assessed in previous studies. BMI and smoking are risk factors forbreast cancer in postmenopausal women [34, 36, 58]. Given weight loss and potential anti-cancer benefits of metformin, physicians may preferably prescribe metformin to overweight or obese patients, as well as smokers. Thus, unmeasured confounding by BMI and smoking is of concern and merits investigation although it would not lead to a spurious protective effect.

Observational studies are useful to evaluate drug safety and effectiveness in real world setting, as well as for hypothesis generation [59, 60]. If incorrectly designed, however, they can suffer from various types of biases leading to spurious results. For example, observational findings on benefits of statins in patients with COPD were recently disproved by a randomized trial [61]. The discordance between observational studies and randomized trials is often portrayed as being the results of a fatal flaw inherent to observational studies, but such a view ignores the fact that not all observational studies are created equal. Observational studies need to be designed using rigorous methods to reduce the potential for bias. Our objective was to investigate whether metformin reduces the risk of breast cancer in a large, nationally representative older population in the US, by conducting a state-of-the art new user cohort study with an active comparator.

II. Methods

Study Population

We included women aged 65 years or older with continuous enrollment in Medicare Parts A, B, and D fee-for-service coverage and no managed care coverage for ≥6 months during the period from 2007 to 2011 and who newly initiated treatment with metformin or sulfonylureas.

Initiation was defined as not receiving any anti-diabetic treatment within 6 months prior to initiation of monotherapy with metformin or sulfonylureas and having ≥1 refill within 90 days after the end of days-supply of the first prescription. The date of the first refill was defined as the index date. Patients were excluded if they had a prior diagnosis of renal disease or cancer during the 6 months before the index date. Patients with renal disease were excluded because metformin is contraindicated in these patients [10]. The flowchart of study population is shown in Figure 4.1.

Follow-up for Breast Cancer

The outcome of interest was a diagnosis of incident breast cancer during follow-up, including both in situ and invasive breast cancer, identified by having at least two ICD-9 diagnosis codes for breast cancer on different dates within 60 days. The date of the first diagnosis was used to define the outcome date. This algorithm has been previously validated in a Medicare population [44].

We used both as-treated (AT; primary) and intention-to-treat (ITT; secondary) analyses. Because breast cancer has a long preclinical phase, we assumed a 180-day induction period for cancer pathogenesis and a 180-day carry-over effect or latency period for cancer detection in the analysis. In the AT approach, follow-up started on 180 days after the index date, and ended with the earliest of the following events: 180 days (latency period) after treatment change or discontinuation, any cancer diagnosis except for non-melanoma skin cancer, death, enrollment gap in Medicare part ABD enrollment greater than 1 month, or end of study (December 31st, 2011). Treatment change was defined as a subsequent addition of or switch to other anti-diabetic drug classes to the index prescription. Treatment discontinuation was defined as no further refill

within the days of supply plus a 90-day grace period. Secondly, the ITT analysis followed patients from 180 days after the index date and until the date of any cancer diagnosis except for non-melanoma skin cancer, death, or end of study, irrespective of any treatment change or discontinuation.

Confounding control

We used propensity scores (PSs) to control measured confounding [46]. For each patient, the probability of receiving metformin vs sulfonylureas was estimated using a logistic regression model, which included demographic and clinical variables that we identified as potential confounders or risk factors for breast cancer. All covariates were defined based on available information during the 6-month period prior to initiation. We standardized the distribution of these covariates to that of the metformin initiators using weights of 1 for metformin initiators and the odds of PS for sulfonylureas initiators [47].

Statistical Analysis

Baseline characteristics were summarized by study cohort and were further adjusted by PS weighting. For each cohort, we used Kaplan-Meier methods to plot cumulative incidence and Poisson regression models to estimate the crude and weighted incidence rates for breast cancer. We then used a Cox proportional regression model to estimate the crude and weighted hazard ratios (HRs) of breast cancer with 95% confidence intervals (CIs) using a robust variance estimation for the weighted model. To explore potential trends of the HRs over time, we estimated HRs in sequential 6-month intervals since the index date. We also performed subgroup analyses, stratified by age group, race, and baseline use of statins.

Several sensitivity analyses were pre-planned. First, given the unresolved concerns whether sulfonylureas have effect on breast cancer risk, we compared the risk of breast cancer in new users of metformin vs two different active comparator groups: (1) new users of thiazolidinediones (TZDs) or incretins, both of which are also oral hypoglycemic agents; (2) diabetic patients who initiated angiotensin-converting-enzyme inhibitors (ACEI) without prior use of any anti-diabetic drugs. Secondly, to minimize the potential misclassification in defining treatment use during follow-up and diabetic patients, we repeated the main study with a longer grace period of 180 days and restricting to new users who had a diagnosis code for diabetes within 6 months before initiation, respectively. Due to undercoding of renal disease, we conducted the analyses including prior renal disease and excluding only those patients with severe renal disease (i.e., chronic kidney disease stage 4 and 5). Additionally, we restricted the outcome of interest to invasive breast cancer only. Lastly, to evaluate the robustness of the assumptions of induction and latency periods, the main analysis was repeated while varying the induction period from 0 to 365 days (for both AT and ITT analysis) and the latency period from 0 to 730 days (for AT analysis).

External Validation Study

To quantify the extent of residual confounding by BMI and smoking that are unavailable in Medicare claims, we conducted a cross-sectional study using external data from the MCBS 2006-2009 panels to identify women initiating metformin or sulfonylureas. New use was defined as initiation of monotherapy with metformin or sulfonylureas after at least 6 months without a prescription for metformin or sulfonylureas. Given the sample size of the MCBS is relatively modest and therefore that the absolute number of women initiating these drugs is small in the MCBS, initiation was defined by requiring only one prescription. We extracted data on

height, weight, and self-reported smoking status from the MCBS Cost & Use module in the same year of initiation. BMI was calculated by weight (kilogram) divided by height (meter) squared, and was treated as a continuous variable as well as a categorical variable (<25 as normal; ≥25 and <30 as overweight; and ≥30 as obese) . Individual smoking status was grouped into never and ever smoker. History of comorbidity and co-medication at baseline were retrieved from the linked Medicare claims data.

We used multivariable logistic regression models to estimate odds ratios (ORs) for the association of BMI and smoking with the initiation of metformin vs sulfonylureas, controlling for those covariates also controlled for in the main study. We then implemented propensity score calibration (PSC) to correct the effect estimates in the Medicare study for confounding by BMI and smoking [53, 62].

III. Results

We identified 36,367 and 11,730 women who initiated metformin or sulfonylureas who met our inclusion criteria, respectively. Compared with metformin initiators, sulfonylureas initiators were older, had more cardiovascular disease (i.e., congestive heart failure and ischemic heart disease), and were more likely to have been admitted to a hospital and visited an emergency room in the 6 months prior to the index date (Table 4.1). Metformin initiators were more likely to have received a prescription for statins, a mammogram or a lipid test compared with sulfonylureas initiators. The characteristics of the women initiating sulfonylureas became very similar to those initiating metformin after PS weighting.

In our primary, AT analysis, 338 patients were diagnosed with breast cancer over 53,271 person-years of follow-up: 262 cases were among metformin initiators and 76 cases were in

sulfonylureas initiators (Table 4.2). The crude incidence rates of breast cancer per 100,000 person-years were 640 (95% CI: 567 to 723) and 615 (95% CI: 491 to 771) in metformin and sulfonylureas initiators, respectively. After PS weighting, the incidence rate was 607 (95% CI: 549 to 670) in sulfonylureas initiators and stayed the same in metformin initiators. The weighted HR comparing metformin with sulfonylureas initiators was 1.08 (95% CI: 0.81 to 1.44) (Table 2). The effect estimate from the ITT analysis was unchanged (adjusted HR: 1.08; 95% CI: 0.86 to 1.35). There was no difference in the cumulative incidence of developing breast cancer by treatment group (Figure 4.2). In Figure 4.3, we examined the risk of breast cancer associated with metformin stratified by duration of treatment after initiation. No decreasing trend was observed after initiation and HR estimates were all close to the null.

Figure 4.4 shows the breast cancer risk for metformin vs sulfonylureas initiators across several subgroups. There was no indication of a protective effect across the age groups and in either subgroup defined by prior statin use. However, we observed a possibly reduced risk for breast cancer associated with metformin in African American women (HR: 0.61; 95% CI: 0.30 to 1.25, for AT) but the confidence interval is wide due to the small number of events. The results were similar in the AT and ITT analyses. We also conducted several sensitivity analyses, showing that metformin was not associated with a lower risk of breast cancer consistently through all scenarios (Tables 4.3, 4.4 and 4.5).

We further controlled for unmeasured confounding by BMI and smoking with PSC. A total of 118 and 79 female initiators of metformin and sulfonylureas were identified from the MCBS. Being obese (BMI: ≥30) and ever smokers were associated with metformin initiation (Table 4.6). These associations were diluted after multivariable adjustment (mainly because of age effects), indicating little difference in BMI and smoking status conditional on controlling for

other differences. After PSC, the HR for breast cancer comparing metformin vs sulfonylureas was 1.05 (95% CI: 0.80 to 1.39) and 1.05 (95% CI: 0.84 to 1.30) based on AT and ITT analyses, respectively.

IV. Discussion and Conclusions

In this large, population-based study using a state-of-the art new user, active comparator cohort design we found that older women initiating metformin did not have a lower risk for breast cancer than women initiating a therapeutic alternative. Similar results were observed when comparing metformin initiators to initiators of TZD/incretin or to diabetic initiators of ACEI. Despite our observation of a possible tendency towards a lower risk of breast cancer associated with metformin in African American women, our result of no effect was consistent across several subgroup and sensitivity analyses.

A meta-analysis found a decreased risk of breast cancer associated with metformin (OR: 0.83; 95% CI: 0.71 to 0.97) and a stronger association with longer metformin use (>3 years) (OR: 0.75; 95% CI: 0.62 to 0.91), subsequently supported by another meta-analysis (OR: 0.94; 95% CI: 0.91 to 0.99) [6, 33]. However, Suissa and Azoulay have pointed out that many of the studies included in the meta-analysis were affected by time-related biases, including immortal time bias, time-window bias, and time-lag bias [7]. In both meta-analyses, for example, the beneficial effect of metformin on breast cancer risk was mainly driven by the case-control study based on data from the Clinical Practice Research Datalink (CPRD), showing that the long-term use of metformin (≥ 40 prescriptions) had a strong effect on preventing breast cancer, compared with no use of metformin (OR: 0.42; 95% CI: 0.21 to 0.87) [1]. This apparent protective effect is likely due to time-window bias, which results from unequal lengths of follow-up time between

cases and controls to define exposure because cases and controls were not matched on time since onset of diabetes or since the first antidiabetic prescription in this study [63]. Among those studies not affected by time-related bias, the findings were controversial: two studies reported protective effects [3, 5] while two reported no effects [29, 30]. In a cohort study from the Netherlands, the risk of breast cancer slightly decreased in new users of metformin compared with new users of sulfonylureas (HR: 0.95; 95% CI: 0.91 to 0.98) [5]. This study included women age 18 or older, a younger study population than ours. Metformin might act differently on breast cancer between pre- and post-menopausal women. The Women Health Initiative (WHI) study found a reduced risk of invasive breast cancer associated with metformin in post-menopausal women (HR: 0.75; 95% CI: 0.57 to 0.99) [3]. Drug exposure in the WHI study was self-reported and collected through questionnaire with unequal intervals whereas our study used data on pharmacy dispensed prescriptions that provide more accurate drug exposure information. Two cohort studies showed evidence of no effect of metformin versus sulfonylureas risk of breast cancer in the UK CPRD (HR: 1.04; 95% CI: 0.79 to 1.37 [30], HR: 1.04; 95% CI: 0.83 to 1.31 [29]).

Our findings suggest that metformin may be associated with a lower risk of breast cancer among African American women, although this association was not statistically significant. African Americans are more likely to develop triple receptor-negative breast cancer (TNBC) than white women [48, 49]. One cohort study of 130 patients with TNBC found that use of metformin was associated a lower risk of distant metastases (HR: 0.61; 95% CI: 0.33 to 1.15) [64], supported by preclinical studies [65, 66]. One plausible explanation for these findings is that metformin may have a favorable effect on TNBC which is more prevalent in African Americans. In the WHI study, metformin use was associated with a greater reduced risk of breast

cancer negative for HER2 overexpression (HR: 0.58; 95% CI: 0.40 to 0.84), compared with overall invasive breast cancer (HR: 0.75; 95% CI: 0.57 to 0.99), despite the fact that the two CIs overlapped [3]. Our subgroup analysis is limited by the small number of breast cancers in African American women observed, thus chance is a plausible alternative explanation.

We used external information from the MCBS to quantify the unmeasured confounding by BMI and smoking on the association between metformin and breast cancer incidence. Obesity and smoking were associated with higher odds of receiving metformin vs sulfonylureas. However, these associations became weak after adjusting for other variables in the PS model, indicating a little independent effect of BMI and smoking of metformin prescribing relative to sulfonylureas and little residual confounding by BMI and smoking on the association between metformin and breast cancer incidence. This lack of effect on relative prescribing given the indication to initiate treatment with oral anti-diabetics is a direct result of the state-of-the art new user, active comparator cohort design [67]. We consistently observed no metformin-breast cancer associations after implementing PSC.

Our study has limitations. It is limited by the short follow-up time (up to 4.5 years). Diabetes treatment regimens are usually modified over time for adequate glycemic control as diabetes progresses, so the observed duration on the initial treatment is limited by actual treatment dynamics (median: 0.79 year; IQR: 0.35 to 1.65) in the AT analysis. In the ITT analysis which ignored treatment changes during follow-up, the follow-up time was almost double (median: 1.53 years; IQR: 0.69 to 2.56), but still short for evaluating a cancer outcome. We thus cannot exclude the possibility of a beneficial effect of long-term use of metformin on breast cancer risk. Due to a relative short period (180 days) of assessment of prior drug use and covariates, our study population may include some patients with prior treatment or prevalent

breast cancer, but we would not expect this to be differential between metformin and sulfonylureas groups. In addition, confounding by unmeasured variables may still exist. We examined the impact of two major unmeasured confounders, BMI and smoking, however, suggesting little residual confounding. Unmeasured confounding by e.g., alcohol consumption or family history of breast cancer would need to be independent of the other covariates that we controlled for. Lastly, we did not independently validate the diagnoses of breast cancer, but our algorithm based on ICD-9 codes has been validated with cancer registry data in an, albeit selected, Medicare population [44].

In conclusion, our findings suggest that metformin does not reduce the risk for breast cancer among women aged 65 years or older. We acknowledge that this study was limited by a short treatment and follow-up time, the former mainly a function of real-world treatment dynamics. Randomized clinical trials have been initiated to evaluate metformin's benefit on cancer incidence and outcomes and will provide more definitive answers; however, this approach may not represent the optimal use of scarce resources, given that the observational evidence leading to these trials likely suffered from avoidable biases.

V. Tables and Figures

TABLE 4.1. Characteristics in New Users of Metformin and Sulfonylureas at Baseline

Characteristics	Metformin	Sulfonylureas	Weighted Sulfonylureas*
Total	36367 (100.0)	11730 (100.0)	(100.0)
Median of Age (IQR)	72.0 (68.0-78.0)	76.0 (70.0-84.0)	72 (67.0-78.0)
Race			
White	13249 (36.4)	2931 (25.0)	(37.2)
African American	9393 (25.8)	2109 (18.0)	(23.7)
Others	6395 (17.6)	2056 (17.5)	(17.2)
Comorbidity			
Benign Breast Disease	1284 (3.5)	290 (2.5)	(3.3)
Benign neoplasm of breast	55 (0.2)	15 (0.1)	(0.1)
COPD	2737 (7.5)	1136 (9.7)	(8.0)
Congestive Heart Failure	3199 (8.8)	2036 (17.4)	(9.2)
Ischemic Heart Disease	6522 (17.9)	2987 (25.5)	(18.4)
Hypertension	28332 (77.9)	9139 (77.9)	(77.9)
Osteoporosis	4069 (11.2)	1259 (10.7)	(11.3)
Medications			
Estrogen	2232 (6.1)	491 (4.2)	(5.9)
Progestin	262 (0.7)	45 (0.4)	(0.7)
Statins	20268 (55.7)	5413 (46.1)	(55.2)
Bisphosphonates	4384 (12.1)	1184 (10.1)	(12.2)
ACE Inhibitors	13715 (37.7)	4354 (37.1)	(37.7)
ARBs	7762 (21.3)	2253 (19.2)	(21.6)
Beta Blockers	14412 (39.6)	4978 (42.4)	(39.6)
Antidepressants	10313 (28.4)	3385 (28.9)	(28.5)
Digoxin	1682 (4.6)	998 (8.5)	(4.8)
Calcium Channel Blockers	10479 (28.8)	3676 (31.3)	(29.1)
Loop Diuretics	5703 (15.7)	2987 (25.5)	(16.2)
Non-Loop Diuretics	14747 (40.6)	3968 (33.8)	(39.9)

TABLE 4.1. *(Continued)* Characteristics in New Users of Metformin and Sulfonylureas at Baseline

Characteristics	Metformin	Sulfonylureas	Weighted Sulfonylureas*
Health Care Use			
Days of hospitalization			
Mean (SD)	0.8 (4.2)	1.6 (5.8)	0.9 (8.0)
Category			
0	32421 (89.1)	9544 (81.4)	(88.7)
1 to 7	2859 (7.9)	1408 (12.0)	(8.2)
7 to 14	577 (1.6)	434 (3.7)	(1.7)
>14	510 (1.4)	344 (2.9)	(1.5)
Number of ER Visit			
0	29544 (81.2)	8596 (73.3)	(80.9)
1	4694 (12.9)	1990 (17.0)	(12.9)
2+	2129 (5.9)	1144 (9.8)	(6.2)
Number of Physician Visit			
0	2462 (6.8)	1237 (10.5)	(7.1)
1-3	9115 (25.1)	3073 (26.2)	(25.4)
4-6	9118 (25.1)	2729 (23.3)	(24.8)
7-12	9794 (26.9)	2976 (25.4)	(26.4)
13+	5878 (16.2)	1715 (14.6)	(16.3)
Mammography	7334 (20.2)	1526 (13.0)	(20.0)
Lipid Test	24905 (68.5)	6439 (54.9)	(67.7)

Abbreviation: IQR: Interquartile Range; ACE inhibitor: angiotensin-converting-enzyme inhibitor; ARB: angiotensin receptor blockers; COPD: Chronic Obstructive Pulmonary Disease; ER: Emergency Room.

* Weighted by standardizing to their distribution in metformin initiators by using weights of 1 for metformin initiators and the odds of the estimated PS for sulfonylureas initiators. PS model includes age in years (continuous variable), race (white, black, and others), comorbidity (Yes/No; benign breast disease, benign neoplasma of breast, chronic obstructive pulmonary disease, chronic heart failure, chronic kidney disease, acute kidney injury, ischemic heart disease, hypertension, and osteoporosis), medication use (Yes/No; estrogen, progestin, statins, bisphosphonates, ACE inhibitors, ARBs, beta blockers, antidepressant, digoxin, calcium channel clockers, loop diuretics, and non-loop diuretics), and healthcare utilization (days of hospitalization (continuous variable), number of physician visit (categorical variable), number of emergency room visit (categorical variable), mammograms (Yes/No), and lipid tests (Yes/No)).

TABLE 4.2. Incidence Rates and Hazard Ratios for Breast Cancer by Treatment Cohort

Cohort	N	BC event	Follow-up Time (years)		Rate per 100,000 pyrs†		HR (95% CI)	
			Sum	Median (IQR)	Crude	PS Weighted*	Crude	PS Weighted*
As-Treated Analysis								
MET	36367	262	40921	0.80 (0.36-1.68)	640 (567-723)	640 (567-723)	1.04 (0.81-1.34)	1.08 (0.81-1.44)
SUL	11730	76	12350	0.73 (0.33-1.55)	615 (491-771)	607 (549-670)	1.00	1.00
Intention-to-Treat Analysis								
MET	36367	395	60311	1.51 (0.68-2.54)	655 (593-723)	655 (593-723)	1.04 (0.85-1.27)	1.08 (0.86-1.35)
SUL	11730	127	20154	1.60 (0.70-2.63)	630 (530-750)	592 (519-675)	1.00	1.00

Abbreviation: MET: metformin; SUL: sulfonylureas; BC: breast cancer; IQR: Interquartile Range; pyrs: person-years; PS: propensity score.

*PS weighted HR were standardized to the distribution of baseline covariates in metformin initiators

† Based on Surveillance, Epidemiology, and End Results Program (SEER) 2007-2011 data, the incidence rate of breast cancer for women aged 65 and over women is 420.5 cases per 100,000 person-years . We observed approximately 1.5-fold incidence rate of breast cancer in the initiators of metformin and sulfonylureas, likely explained by the diabetic study population in our study.

TABLE 4.3. Sensitivity Analyses

Sensitivity Analysis	Analysis	Cohort	N	BC event	Median of Follow-up years (IQR)	Rate per 100,000 person-years	PS Weighted HR (95% CI)*
Using initiators of TZD/Incretin as reference	ITT	MET	36397	395	1.51 (0.68-2.54)	655 (593-722)	1.08 (0.78-1.49)
		TZD/DPP	4533	48	1.72 (0.75-2.77)	592 (446-786)	1.00
	AT	MET	36397	262	0.80 (0.36-1.68)	640 (567-722)	0.91 (0.60-1.38)
		TZD/DPP	4533	27	0.61 (0.33-1.33)	629 (431-917)	1.00
Using diabetic initiators of ACEI as reference	ITT	MET	18913	201	1.52 (0.69-2.55)	639 (557-734)	1.00 (0.79-1.26)
		ACEI	20583	209	1.58 (0.73-2.57)	598 (522-685)	1.00
	AT	MET	18913	114	0.68 (0.33-1.43)	608 (506-731)	0.96 (0.69-1.32)
		ACEI	20583	117	0.70 (0.34-1.48)	549 (458-657)	1.00
Initiators with a prior DM diagnosis	ITT	MET	32450	335	1.53 (0.70-2.56)	619 (556-689)	1.03 (0.81-1.29)
		SUL	10925	119	1.63 (0.72-2.66)	629 (525-753)	1.00
	AT	MET	32450	222	0.81 (0.36-1.69)	604 (530-689)	1.03 (0.77-1.39)
		SUL	10925	72	0.74 (0.33-1.57)	622 (493-783)	1.00
Outcome including invasive breast cancer only	ITT	MET	36442	390	1.52 (0.69-2.55)	643 (583-711)	1.05 (0.84-1.32)
		SUL	11761	128	1.61 (0.71-2.64)	632 (531-751)	1.00
	AT	MET	36442	260	0.81 (0.36-1.68)	632 (560-714)	1.05 (0.79-1.40)
		SUL	11761	78	0.73 (0.33-1.56)	629 (504-785)	1.00

TABLE 4.3. *(Continued)* Sensitivity Analyses

Sensitivity Analysis	Analysis	Cohort	N	BC event	Median of Follow-up years (IQR)	Rate per 100,000 person-years	PS Weighted HR (95% CI)*
Including patients with or without history of renal disease	ITT	MET	38435	405	1.51 (0.68-2.53)	638 (578-703)	1.06 (0.86-1.31)
		SUL	14558	149	1.53 (0.66-2.57)	611 (520-718)	1.00
	AT	MET	38435	269	0.80 (0.35-1.66)	627 (557-707)	1.12 (0.85-1.47)
		SUL	14558	87	0.70 (0.33-1.50)	581 (471-717)	1.00
Excluding patients with history of chronic kidney disease stage 4-5	ITT	MET	38251	404	1.51 (0.68-2.54)	639 (579-704)	1.05 (0.85-1.30)
		SUL	13803	144	1.54 (0.67-2.59)	619 (526-729)	1.00
	AT	MET	38251	269	0.80 (0.35-1.66)	629 (558-709)	1.09 (0.83-1.43)
		SUL	13803	85	0.71 (0.33-1.51)	595 (481-736)	1.00
Grace Period of 180 days	ITT	MET	36387	397	1.51 (0.68-2.55)	657 (595-725)	1.09 (0.87-1.37)
		SUL	11460	123	1.62 (0.71-2.66)	621 (520-741)	1.00
	AT	MET	36387	301	0.96 (0.44-1.87)	667 (596-747)	1.20 (0.90-1.59)
		SUL	11460	79	0.86 (0.38-1.75)	594 (477-741)	1.00

Abbreviation: AT: as-treated; ITT: intention-to-treat; MET: metformin; SUL: sulfonylureas; TZD: Thiazolidinediones; ACEI: angiotensin-converting-enzyme inhibitors; BC: breast cancer; IQR: Interquartile Range; pyrs: person-years; PS: propensity score.

* Propensity score (PS) was re-estimated based on the same model for each sensitivity analysis.

TABLE 4.4. Sensitivity Analyses for Induction and Latency Periods in As-Treated Analysis

Induction Period (days)	Latency Period (days)	Cohort	N	BC event	Median of Follow-up years (IQR)	Rate per 100,000 pyrs	PS Weighted HR (95% CI)*
0	0	MET	43427	414	0.84 (0.40-1.77)	791 (718-871)	1.16 (0.92-1.47)
		SUL	13958	107	0.75 (0.33-1.63)	689 (570-832)	1.00
90	0	MET	37336	304	0.78 (0.29-1.72)	716 (640-802)	1.19 (0.90-1.58)
		SUL	11593	77	0.71 (0.27-1.61)	620 (496-775)	1.00
180	0	MET	29404	222	0.87 (0.33-1.80)	650 (570-741)	1.10 (0.80-1.52)
		SUL	8947	60	0.79 (0.30-1.69)	607 (471-782)	1.00
360	0	MET	19067	144	0.93 (0.41-1.79)	650 (552-765)	1.15 (0.77-1.73)
		SUL	5566	37	0.90 (0.38-1.73)	588 (426-811)	1.00
0	90	MET	43427	441	0.96 (0.57-1.85)	778 (709-854)	1.14 (0.91-1.43)
		SUL	13958	117	0.89 (0.51-1.74)	681 (568-816)	1.00
90	90	MET	39745	328	0.82 (0.39-1.72)	706 (634-787)	1.17 (0.89-1.52)
		SUL	12813	86	0.73 (0.33-1.59)	619 (501-764)	1.00
180	90	MET	34168	240	0.77 (0.28-1.68)	642 (566-729)	1.12 (0.82-1.52)
		SUL	10616	65	0.70 (0.26-1.57)	589 (462-751)	1.00
360	90	MET	20883	154	0.90 (0.38-1.75)	649 (554-760)	1.16 (0.78-1.72)
		SUL	6253	40	0.87 (0.35-1.69)	583 (428-795)	1.00
0	180	MET	43427	466	1.08 (0.66-1.93)	770 (703-843)	1.13 (0.91-1.40)
		SUL	13958	128	1.03 (0.63-1.83)	686 (577-815)	1.00
90	180	MET	39745	353	0.95 (0.55-1.80)	702 (632-779)	1.14 (0.89-1.47)
		SUL	12813	97	0.87 (0.50-1.68)	631 (517-770)	1.00
180	180	MET	36367	262	0.80 (0.36-1.68)	640 (567-723)	1.08 (0.81-1.44)
		SUL	11730	76	0.73 (0.33-1.55)	615 (491-771)	1.00
360	180	MET	23320	161	0.85 (0.35-1.69)	629 (539-734)	1.08 (0.75-1.56)
		SUL	7178	47	0.80 (0.32-1.63)	623 (468-829)	1.00
0	360	MET	43427	510	1.38 (0.76-2.12)	761 (698-830)	1.18 (0.96-1.46)
		SUL	13958	137	1.34 (0.79-2.06)	647 (547-764)	1.00
90	360	MET	39745	397	1.23 (0.70-1.99)	699 (634-771)	1.21 (0.96-1.54)
		SUL	12813	106	1.19 (0.73-1.91)	592 (490-717)	1.00
180	360	MET	36367	306	1.05 (0.61-1.85)	646 (577-722)	1.18 (0.90-1.54)
		SUL	11730	85	1.02 (0.59-1.77)	572 (462-707)	1.00
360	360	MET	29200	196	0.78 (0.37-1.58)	636 (553-732)	1.26 (0.90-1.77)
		SUL	9507	52	0.74 (0.33-1.50)	548 (418-720)	1.00
0	720	MET	43427	567	1.68 (0.76-2.51)	754 (694-818)	1.12 (0.92-1.36)
		SUL	13958	166	1.77 (0.79-2.54)	678 (582-789)	1.00
90	720	MET	39745	454	1.60 (0.70-2.35)	699 (637-766)	1.13 (0.91-1.40)
		SUL	12813	135	1.66 (0.73-2.37)	637 (538-754)	1.00
180	720	MET	36367	363	1.51 (0.68-2.18)	653 (589-724)	1.09 (0.86-1.37)
		SUL	11730	114	1.55 (0.70-2.20)	627 (522-754)	1.00
360	720	MET	29200	253	1.29 (0.66-1.86)	649 (573-734)	1.09 (0.83-1.44)
		SUL	9507	81	1.31 (0.72-1.85)	634 (510-788)	1.00

TABLE 4.5. Sensitivity Analyses for Induction Periods in Intention-to-Treat Analysis

Induction Period (days)	Cohort	N	BC event	Median of Follow-up years (IQR)	Rate per 100,000 pyrs	PS Weighted HR (95% CI)*
0	MET	43427	599	1.68 (0.76-2.83)	749 (692-812)	1.11 (0.92-1.34)
	SUL	13958	179	1.77 (0.79-2.90)	676 (584-783)	1.00
90	MET	39745	486	1.60 (0.70-2.66)	697 (638-762)	1.12 (0.91-1.37)
	SUL	12813	148	1.66 (0.73-2.76)	638 (543-750)	1.00
180	MET	36367	395	1.51 (0.68-2.54)	655 (593-723)	1.08 (0.86-1.35)
	SUL	11730	127	1.60 (0.70-2.63)	630 (530-750)	1.00
360	MET	29200	285	1.41 (0.66-2.30)	652 (580-732)	1.08 (0.83-1.40)
	SUL	9507	94	1.51 (0.72-2.38)	636 (520-779)	1.00

Abbreviation: MET: metformin; SUL: sulfonylureas; BC: breast cancer; IQR: Interquartile Range; pyrs: person-years; PS: propensity score.

*Propensity score (PS) was re-estimated based on the same model for each sensitivity analysis

TABLE 4.6. Characteristics in Metformin and Sulfonylureas at Baseline in the MCBS 2006-2009

Characteristics	MET	SUL	Crude OR (95% CI)	Adjusted OR (95% CI)[#]
Total	118 (100.0)	79 (100.0)		
Median Age (IQR)	74.0 (70.0-80.0)	78.0 (75.0-84.0)	0.92 (0.88-0.96)[$]	0.94 (0.89, 0.99)
Race				
White	89 (75.4)	59 (74.7)	1.04 (0.54-2.01)	0.85 (0.40, 1.82)
Other	29 (24.6)	20 (25.3)	1.00	1.00
Median of BMI (IQR)	29.9 (25.6-34.0)	28.6 (25.1-33.1)	1.01 (0.97-1.06)[$]	--
Mean of BMI (SD)	30.5 (6.5)	29.9 (6.9)		
BMI Category*				
<25	24 (20.3)	18 (22.8)	1.00	1.00
25 to <30	35 (29.7)	30 (38.0)	0.87 (0.40-1.91)	0.84 (0.34, 2.06)
≥30	58 (49.2)	29 (36.7)	1.50 (0.70-3.20)	1.27 (0.52, 3.10)
Smoking Status*				
Never	61 (51.7)	48 (60.8)	1.00	1.00
Ever Smoking	57 (48.3)	28 (35.4)	1.60 (0.89-2.89)	1.41 (0.72, 2.74)

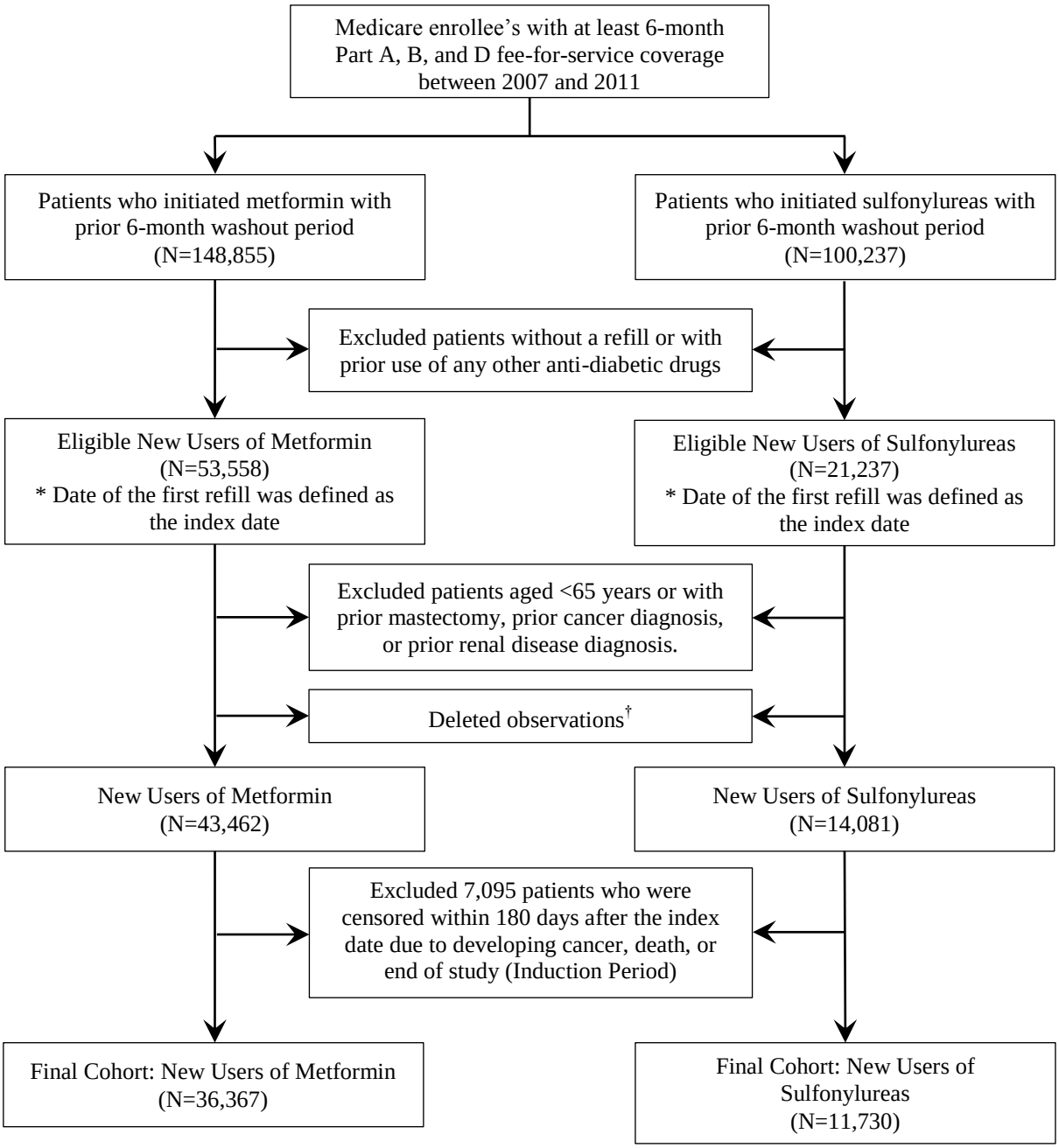
Abbreviation: MET: metformin initiators; SUL: sulfonylureas initiators; IQR: interquartile range; SD: standard deviation

* Missing data on BMI and Smoking status were less than 5%. Our DUA does not allow us to present cell sizes <11, so the number of missing was not presented on this table.

$ OR for 1 unit increase

Adjusted OR was controlled for BMI (categorical), smoking status (never and ever), age, race (white and others), congestive heart failure, ischemic heart disease, beta blocker, anti-hypertensive drugs, loop diuretics, mammogram, admission to hospital, and physician visit in the PS model, as known as gold-standard PS in PSC method.

† To implement PSC, two PSs were estimated within the MCBS data: the error-prone PS ($PS_{EP, MCBS}$) based on covariates available in claims, and the gold-standard PS ($PS_{GS, MCBS}$) based on BMI and smoking status in addition to the variables available in claims. We fit a model for the simple linear regression of $PS_{GS, MCBS}$ on $PS_{EP, MCBS}$ and treatment. Then, based on the estimated parameters from this model, we imputed a new PS (considered as "Gold-standard", $PS_{GS, Medicare}$) with the original PS (considered as "error-prone", $PS_{EP, Medicare}$) and treatment in the main study. The association of metformin-breast cancer was estimated in a Cox model, weighted by imputed $PS_{GS, Medicare}$. We acknowledge that the surrogacy assumption for PSC may be violated in our study, but we were not able to test this assumption due to lack of outcome information in the MCBS. However, our results showed a little residual unmeasured confounding by BMI and smoking, thus leading to an unbiased PSC.



† One patient can contribute to multiple observations of new use, if applicable. Only the first observation for each patient was included in the final study cohort

FIGURE 4.1. The flowchart of the study population

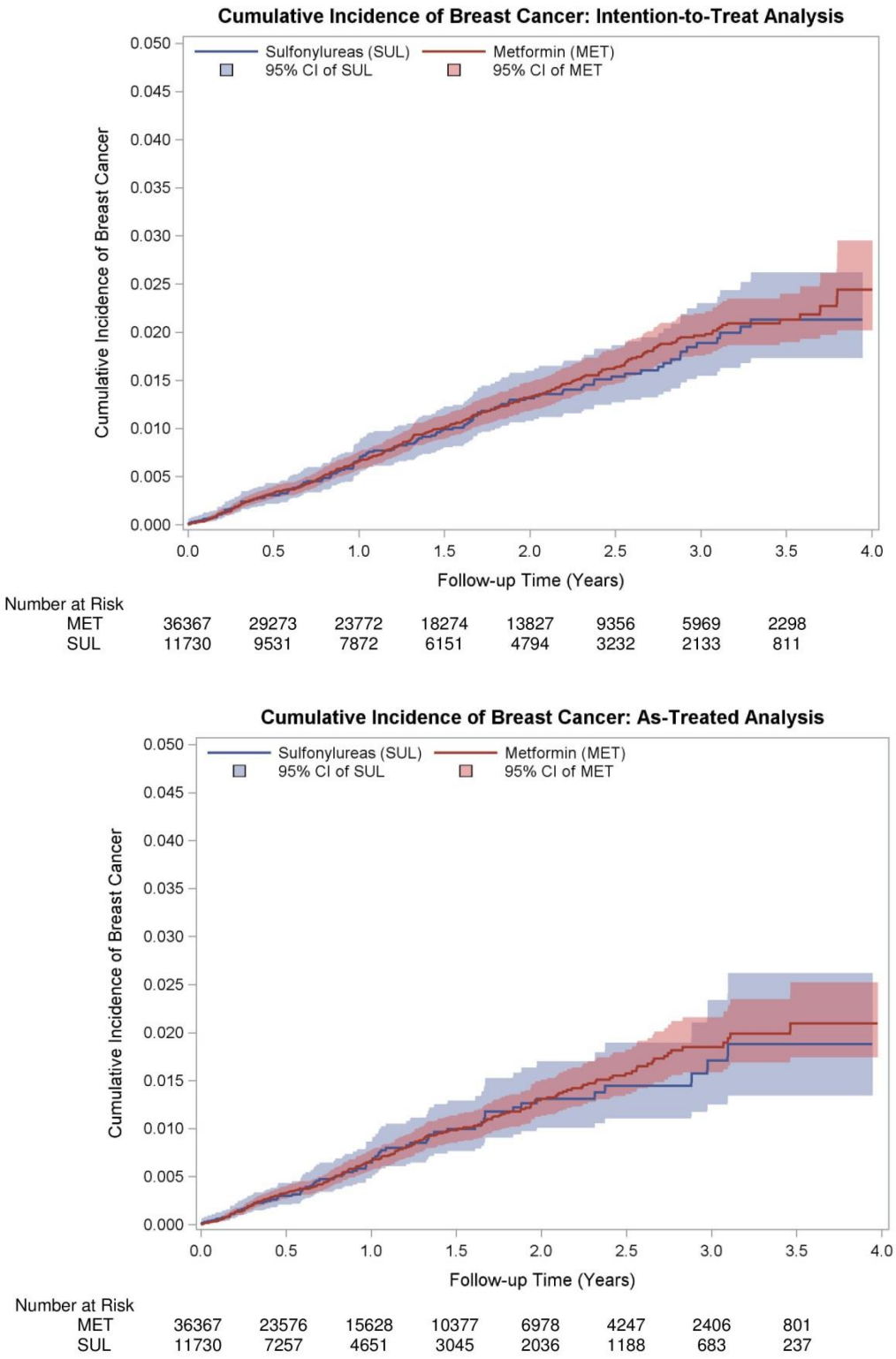
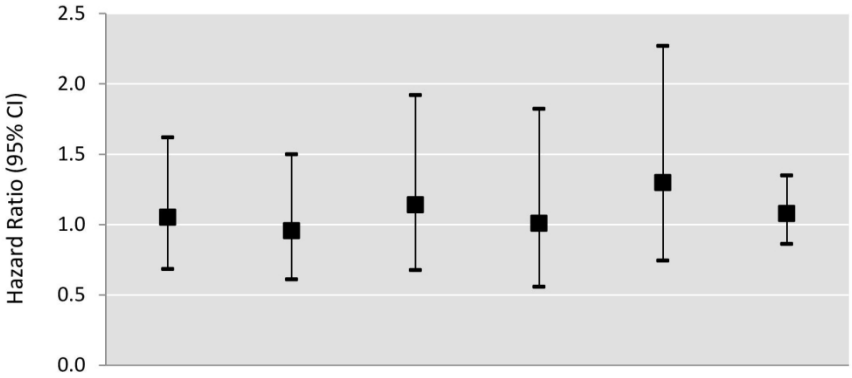
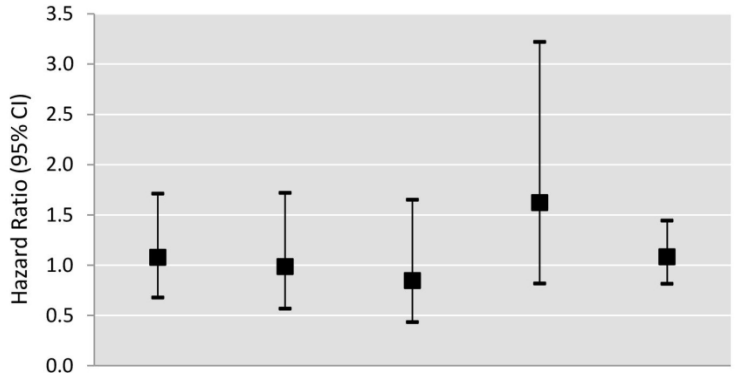


FIGURE 4.2. Cumulative Incidence of Breast Cancer by Treatment Cohort.

The number of patients at risk at year 4 was not listed because our DUA prohibits us to present number less 11.



Follow-up (months)	0-6	7-12	13-18	19-24	25+	Overall
Initiators, n						
Metformin	36367	29273	23772	18274	13827	36367
Sulfonylureas	11730	9531	7872	6151	4797	11730
Breast Cancer Cases, n						
Metformin	108	87	74	52	74	395
Sulfonylureas	33	31	23	18	22	127
Estimates						
HR	1.05	0.96	1.14	1.01	1.30	1.08
95% Lower Limit	0.68	0.61	0.68	0.56	0.74	0.86
95% Upper Limit	1.62	1.50	1.92	1.82	2.27	1.35

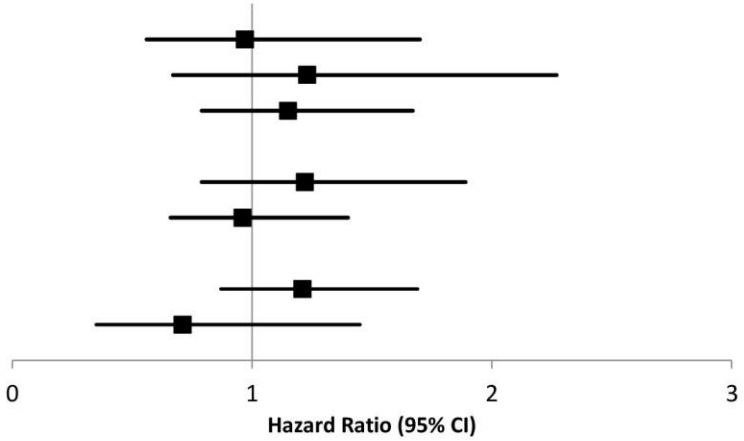


Follow-up (months)	0-6	7-12	13-18	18+	Overall
Initiators, n					
Metformin	36367	23576	15628	10377	36367
Sulfonylureas	11730	7257	4651	3045	11730
Breast Cancer Cases, n					
Metformin	100	63	42	57	262
Sulfonylureas	29	20	14	13	76
Estimates					
HR	1.08	0.99	0.85	1.62	1.08
95% Lower Limit	0.68	0.57	0.43	0.82	0.81
95% Upper Limit	1.71	1.72	1.65	3.22	1.44

FIGURE 4.3. PS weighted hazard ratios (95% CI) over time comparing metformin initiators vs sulfonylureas initiators since follow-up, in as treated (top) and intention to treat (bottom) analyses.

As-Treated Analysis

Subgroup	Metformin Patients/Case	Sulfonylureas Patients/Case	HR (95% CI)
Age of 65-69	13,249/88	2,931/19	0.97 (0.56-1.70)
Age of 70-74	9,393/75	2,109/14	1.23 (0.67-2.27)
Age of 75+	13,725/99	6,690/43	1.15 (0.79-1.67)
Statin	20,268/144	5,413/33	1.22 (0.79-1.88)
No Statin	16,099/118	6,317/43	0.96 (0.65-1.40)
White	28,855/217	9,088/57	1.21 (0.87-1.69)
African American	3,858/23	1,590/13	0.61 (0.30-1.25)



Intention-to-Treat Analysis

Subgroup	Metformin Patients/Case	Sulfonylureas Patients/Case	HR (95% CI)
Age of 65-69	13,249/137	2,931/37	1.00 (0.67-1.49)
Age of 70-74	9,393/110	2,109/28	1.00 (0.64-1.58)
Age of 75+	13,725/148	6,690/62	1.26 (0.92-1.72)
Statin	20,268/213	5,413/51	1.25 (0.88-1.77)
No Statin	16,099/182	6,317/76	0.95 (0.71-1.27)
White	28,855/331	9,088/95	1.20 (0.93-1.56)
African American	3,858/35	1,590/23	0.65 (0.38-1.13)

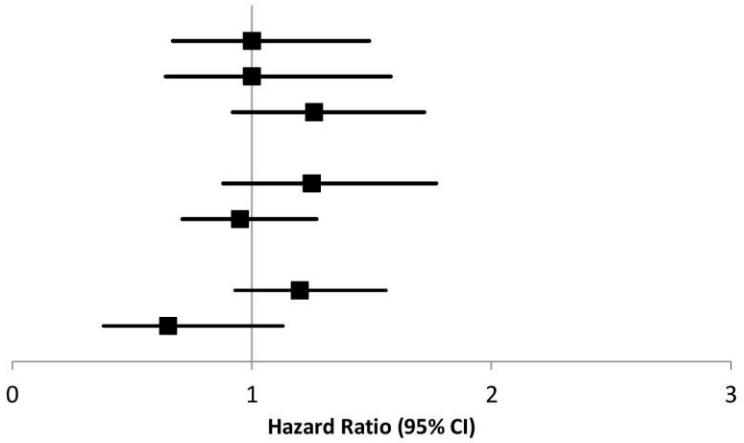


FIGURE 4.4. Propensity Score weighted hazard ratios (95% CIs) comparing metformin initiators vs sulfonylureas initiators, stratified by age group, race, and baseline use of statins, in as treated (top) and intention to treat (bottom) analyses.

*Propensity score was re-estimated based on the same model for each subgroup.

CHAPTER 5. DIFFERENTIAL USE OF SCREENING MAMMOGRAPHY IN OLDER WOMEN INITIATING METFORMIN VERSUS SULFONYLUREAS

I. Introduction

Metformin is the most commonly prescribed drug for type 2 diabetes. It has been linked with possible beneficial effects on breast cancer incidence in several observational studies [1, 3, 5, 32] but not in others [2, 4, 27-30]. Many of the studies reporting an inverse association between metformin and breast cancer risk may suffer from time-related biases as discussed in Suissa's review article [7]. Another potential source of bias, detection bias due to differential healthcare utilization, could also affect the metformin-breast cancer association but has not been addressed so far. Differential detection of asymptomatic or pre-clinical cancer before treatment initiation could reduce cancer incidence after initiation by excluding women with early diagnosis. On the other hand, differential detection after initiation may lead to an increased incidence rate immediately following treatment initiation followed by a period of reduced incidence rate.

Our previous study examined healthcare utilization comparing metformin and a clinical alternative, sulfonylureas, using data from Medicare claims [68]. We have noted that metformin initiators were more likely than sulfonylureas initiators to receive mammograms and to visit physicians around the time of initiation, potentially leading to biased detection of breast cancer. However, this study failed to distinguish screening from diagnostic mammography, thus the results did not reflect the actual difference in receipt of screening mammography between metformin and sulfonylureas initiators. Therefore, the objective of this study was to quantify the

risk of screening mammography and screen-detected breast cancer over 12-month periods pre- and post-initiation among metformin and sulfonylureas initiators.

II. Methods

Study Population

This study used data from Medicare fee-for-service beneficiaries from January 1st, 2006 to December 31st, 2012, including the denominator file, Part A (hospital claims), Part B (outpatient physician services), and Part D (dispensed prescription claims). Medicare offers free screening mammography every year for all women, minimizing the influences of socioeconomic disparity. Given that differential detection pre- and post-initiation would affect the effect estimates for incident breast cancer differently, this study had two study cohorts. One was the new user cohort which was used to examine use of screening mammography over a two-year window of 12 months pre- and post-initiation, primarily focusing on the period of 12 months prior to initiation. The other study cohort was the cancer-free cohort mimicking a cohort study on breast cancer incidence, which only examined use of screening mammography in 12 months after initiation.

For the new user cohort, we identified women aged 65 or older who initiated monotherapy of metformin or sulfonylureas between 2008 and 2010. Initiation was defined as having at least one refill within 90 days after end of drug supply of the initial prescription and having at least 12 months of continuous Part D enrollment prior to initiation without use of any anti-diabetic drugs. Patients were classified as new users of metformin or sulfonylureas according to the initial prescription and the date of the first prescription was defined as the index date (i.e., initiation). Eligible patients were also required to have enrolled in Part A and B

continuously for ≥ 24 months pre-initiation and ≥ 12 months post-initiation, thus patients who died or disenrolled Medicare Part A and B within 12 months after initiation were excluded from the study.

A study examining cancer incidence commonly requires a cancer-free study population. Thus, we had the cancer-free cohort to correctly assess receipt of screening mammography within 12 months following treatment initiation. This cancer-free cohort was a subgroup of the new user cohort, which included eligible initiators without a diagnosis of any cancer except for non-melanoma skin cancer within 12 months prior to initiation. The flowchart of the study cohort is available in Figure 5.1.

Screening Mammography

We used the following the Healthcare Common Procedure Coding System (HCPCS) codes to select all mammograms for the study cohort: G0202-G0205, 76091-76092, 77051-77052, and 77056-77057. Mammograms were further classified as screening versus diagnostic test based on a claims-based algorithm [69]. Briefly, mammograms were considered as screening test if they were coded as screening mammography without a previous mammogram within prior 9 months and without any breast cancer diagnosis in the prior year. This algorithm has been validated in Medicare claims with a high positive predictive value (PPV) of 94.9% [69]. The detail of this algorithm and the results were shown in Supplemental Figures 5.2-5.3.

Screen-Detected Breast Cancer

After distinguishing screening from diagnostic mammograms, we also used the Fenton algorithm to identify incident screen-detected breast cancers [70]. This algorithm has a high PPV of 88.0% among Medicare enrollees [70]. The Fenton algorithm classifies screening

mammograms as positive to detect breast cancer by requiring a breast cancer diagnosis within 123 days post-mammogram and a breast-directed surgery within a year following the diagnosis, or by a diagnosis of carcinoma in-situ within 286 days post-mammogram with a subsequent mammogram within 82 days following the diagnosis. The detail of this algorithm and the results were shown in Figures 5.4-5.5. To evaluate the performance of screening mammography in our cohorts, we calculated the screening detection rate for breast cancer by dividing the number of screen-detected breast cancers by the number of screening mammograms. The corresponding 95% confidence intervals were estimated using the adjusted Wald method.

Incident Breast Cancer

In the cancer-free cohort, any incident breast cancer during 12-month follow-up was another outcome of interest, irrespective of whether it was detected by screening or due to symptoms. To be similar to the Fenton algorithm, we required a breast-directed surgery within a year following a breast cancer diagnosis code, including invasive and carcinoma in situ, to ascertain the breast cancer case.

Statistical Analysis

In the new user cohort, we began with a description of metformin and sulfonylureas initiators by receipt of screening mammography during a two-year window of 12 months pre- and post-initiation. The day of initiation was indexed as Month 0, and was included in the month following initiation (Month 1). Frequency of initiators receiving a screening mammogram was summarized within sequential 3-month and 12-month intervals from Month -12 (before initiation) to Month 12 (after initiation), respectively. Incident breast cancer detected at screening was calculated over 12-monthly intervals, given expected small numbers. We estimated risks and risk

differences (RDs) and their 95% confidence intervals (CIs) of each event during each time interval comparing metformin to sulfonylureas initiators. To control the potential confounding, we used propensity score weighting method to standardize sulfonylureas initiators to metformin initiators on the following variables: age, race, calendar year of initiation, and number of physician visit in 12 months prior to initiation [47].

In the cancer-free study, we repeated all analyses during the time window of 12 months after initiation only and calculated RD of total breast cancer incidence comparing metformin to sulfonylureas groups in 12 months after initiation. Given the fact that screening mammography prior to initiation is likely associated with receiving subsequent screening tests after initiation, we additionally included the variable of prior screening in the propensity score model for confounding control. Furthermore, the analyses in this cancer-free cohort were stratified by receipt of screening mammography in 12 months prior to initiation.

III. Results

Our new user cohort included a total of 36,465 initiators of metformin and sulfonylureas during the study period. Tables 5.1 and 5.2 summarize the baseline characteristics and the proportion of women who had a least 1 screening mammogram in 2 years between metformin and sulfonylureas initiators, respectively. Compared with sulfonylureas initiators, metformin initiators were younger, had less comorbidity such as cancer and cardiovascular disease, and had more physician visits. Patients were less likely to initiate sulfonylureas in more recent years, but were equally likely to initiate metformin over three years. Over a two-year window, 45% of the new user cohort received at least one screening mammogram. Approximately half of metformin initiators but only one-third of sulfonylureas initiators had at least one screening mammogram in

two years (Table 5.2). The proportion of women who had screening mammograms decreased largely with age but increased with number of physician visits in both groups. Screening mammography was also less common in sicker patients, such as those with cardiovascular disease or hospital admission, but remained similar across calendar years.

We present the proportion of patients receiving mammograms in 3-monthly intervals over 12 months before and after initiation in Figure 5.6. Metformin initiators were more likely to have screening mammograms than sulfonylureas initiators, which was evident and consistent over time. For both cohorts, the percentage of patients receiving screening mammograms was fairly stable over time; however, it clearly peaked in the 3 months immediately following initiation in metformin initiators but not in sulfonylureas initiators, resulting in a greater difference of 4 percentage points in this interval than other intervals (approximately 3 percentage points).

We identified a total of 16,788 and 4,537 screening mammograms over two years in 13,220 (50%) metformin initiators and 3,312 (33%) sulfonylureas initiators, respectively (Table 5.3). The weighted RD of screening mammography comparing metformin to sulfonylureas groups was 10 percentage points (95% CI: 9 to 10). We also examined use of screening mammograms before and after initiation, separately (Table 5.4). During 12 months prior to initiation, we found 9,210 (35%) and 2,274 (23%) initiators of metformin and sulfonylureas who had at least one screening mammogram, respectively, resulting in a weighted RD of 7 percentage points (95% CI: 6 to 8). Similar results were found for the analyses during 12 months after initiation (weighted RD: 8 percentage points; 95% CI: 7 to 9).

We found 137 and 23 incident breast cancer was detected at screening mammograms among metformin and sulfonylureas groups, respectively (Table 5.3). The risk of incident breast cancer detected at screen was 0.52% in metformin, as compared with 0.23% in sulfonylureas, with a weighted RD of 0.17 percentage points (95% CI: 0.06 to 0.28). However, a higher absolute risk of screen-detected breast cancer was observed for metformin group during 12 months after initiation (weighted RD: 0.12 percentage points; 95% CI: 0.04 to 0.21), but not during 12 months before initiation (weighted RD: 0.05 percentage points; 95% CI: -0.02 to 0.12) (Table 5.4).

We found similar patient characteristics (Tables 5.5-5.6) and the results of screening mammography and screen-detected breast cancer during 12 months after initiation in the cancer-free study cohort (Tables 5.7). Consistently, metformin initiators were more likely to receive screening mammograms than sulfonylureas initiators (weighted RD: 6 percentage points; 95% CI: 5 to 7). The risk of screen-detected breast cancer was higher in metformin (0.33%) than sulfonylureas (0.13%), but differences became attenuated after accounting for imbalances in baseline characteristics (weighted RD: 0.09 percentage points; 95% CI: -0.01 to 0.19). Although metformin was not associated with the risk of total breast cancer within 1 year after initiation (weighted RD: 0.06 percentage points; 95% CI: -0.07 to 0.19), more breast cancer cases were actually detected by screening in metformin initiators (62%) than sulfonylureas initiators (34%).

We stratified the analyses by receipt of screening mammography in 12 months prior to initiation (Table 5.8). Women screened in previous 12 months were more likely to receive screening mammograms in the following 12 months after initiation than those not screened, consistently in both metformin and sulfonylureas groups. The risk difference in screening mammograms between metformin and sulfonylureas was larger for women not screened

previously than those screened. In addition, the positive association between metformin and higher risks of screen-detected breast cancer and total breast cancer was only found among women not previously screened, but not among women previously screened (Table 5.9). As expected, the screening detection rate for incident breast cancer was higher among women not previously screened.

IV. Discussion and Conclusions

In this study, we found that older women initiating metformin were not only more frequently screened for breast cancer in 12 months before initiation than women initiating sulfonylureas, but they also had more subsequent screening mammograms in the 12 months after initiation, especially those women not screened in the year pre-initiation. Consequently, compared with sulfonylureas initiators, metformin initiators had higher probabilities of screen-detected breast cancer both in the 12 months before and after initiation. These results indicate existence of detection bias due to differential screening mammography pre- and post-initiation when comparing breast cancer incidence between women initiating metformin and women initiating sulfonylureas.

A total of 45% of all eligible initiators in our study underwent at least one screening mammogram over two-year time window, far less than that reported by US Centers for Disease Control and Prevention, which estimated 65.5% of women aged 65 years and over in 2008 having a mammogram within the past 2 years [71]. Nevertheless, our results were similar to the previous study of 5% Medicare random sample, which reported that 40.2% of women aged 65+ years in Medicare during 2005-2006 had at least one screening mammography [72]. We also observed that receipt of screening mammography was associated inversely with age but

positively with number of physician visits. Although the United States Preventive Services Task Force (USPSTF) recommendations on screening mammography changed in 2009, no difference was observed on use of screening mammography across calendar years. These findings are consistent with previous studies [72, 73]. The overall detection rate for screen-detected breast cancer was 6.9 per 1,000 screening examinations in our study, similar to Breast Cancer Surveillance Consortium 2009 data which ranges from 6.1 to 8.5 as age increases from 65 to 85+ .

It is unknown why the use of screening mammography could differ between metformin and sulfonylureas initiators since both groups represented similar diabetic patients newly prescribed with oral anti-diabetic drugs, but we speculate that it could be related to prescriber's behavior. The USPSTF recommends biennial screening mammography for women aged 50-74 years . Previous studies also have shown that physician recommendation is one strong motivation for undergoing screening mammography [75, 76]. Given that metformin is recommended as the preferred initial treatment for diabetes except for patients with chronic kidney disease, metformin prescribers who comply with guideline recommendations may be more likely to perform regular examinations or to recommend cancer screening tests for older patients. Thus, women initiating metformin may be more likely to receive screening mammography and, consequently, to be diagnosed with breast cancer around the time of initiation. While this theory is purely speculative, we observed a sudden increase in the probability of receiving screening mammography in the 3 months after initiation in metformin group but not in sulfonylureas group, indicating a possible role of the prescribers on promoting screening mammography. This finding may provide some support for our speculation.

Differential detection of breast cancer due to differential screening mammography before and after drug initiation has distinct influences on effect estimates, thus our results need to be interpreted separately. Differential screening mammography before initiation which we observed in the new user cohort suggests that metformin initiators may be at slightly lower risk of breast cancer than sulfonylureas initiators at the time of initiation because more asymptomatic breast cancer cases were excluded from metformin group, in spite of the fact that the difference in the probability of screen-detected breast cancer was small.

On the other hand, differential screening mammography after initiation would increase the breast cancer incidence in metformin initiators immediately following treatment initiation and would decrease the breast cancer incidence thereafter because of the "premature" detection of preclinical breast cancer that would eventually become clinical and be diagnosed without screenings. One cohort study examined metformin users in UK clinical practice research datalink (CPRD) and found the pattern of a higher breast cancer risk in the first 6 months since initiation than later [32], supporting our hypothesis. This may also explain the unexpected increased incidence of breast cancer in metformin users which was observed in one cohort study of US Medicare claims [28], and, in addition to time-related bias [7], might also partially explain the benefits of metformin on breast cancer incidence which was only observed after long-term treatment in one CPRD case-control study [1].

Interestingly enough, in cohort studies of cancer incidence in which follow-up begins only 6 or 12 months after drug initiation to account for a likely induction period for cancer development, differential screening after initiation could lead to a relative lower risk of breast cancer for metformin initiators given more cases of screen-detected breast cancer are excluded from metformin initiators than sulfonylureas initiators. Together, our results on differential

screening mammography are unlikely to explain why we should observe no association between metformin in comparison with sulfonylureas and breast cancer as observed in some studies assuming a real protective effect of metformin [27, 29, 30]. Our results might, however, at least partially explain a reduced risk of breast cancer associated with metformin as observed in one cohort study with sulfonylureas as a comparison group [5] and in several studies with different comparison groups [1-3] assuming no effect of metformin on breast cancer incidence.

This study has some limitation. First, our study is limited by the small number of screen-detected breast cancer cases, thus the effect estimates (i.e., RD) were imprecise. We acknowledge that we did not have a large enough study population to detect a small difference in the risk of screen-detected breast cancer during the study period. Secondly, we may have underestimated true cases of breast cancer detected at screening. To ascertain breast cancer cases, we required a breast surgery following a breast cancer diagnosis, according to the Fenton algorithm [69]. Although surgery is the primary and most effective treatment for breast cancer, it is still possible that older and sicker women may not have surgery [77]. Given the limitations due to sample size, we did not use information on prescriber's characteristics to evaluate our speculation that differential screening test may be related to prescriber's behavior. Lastly, Medicare part B plan provides free annual screening mammography for women aged 65 years or older, reducing health inequalities for receiving mammography. Thus, our results can be generalized to US older women, but may not be generalized to younger women or women residing in other countries in which do not provide full coverage of screening mammography for older women and socioeconomic status likely affects the probability of receiving screening mammography. Generalizability of the study results may be limited within Medicare population.

Our study provides empirical evidence for biased detection for breast cancer due to more screening mammograms performed in older women initiating metformin compared with sulfonylureas around the time of initiation. Researchers should be aware of the potential for more screening mammograms pre- and post-initiation when interpreting the findings of studies assessing the effects of metformin on breast cancer incidence.

V. Tables and Figures

TABLE 5.1. Characteristics at baseline among metformin and sulfonylureas initiators in the new user cohort

Characteristics	Metformin	Sulfonylureas
	N (%)	N (%)
Total N	26532 (100.0)	9933 (100.0)
Age, years		
Median	73.0	77.0
Interquartile Range (IQR)	(69.0-79.0)	(71.0-84.0)
Category		
65 - 69	7362 (27.7)	2039 (20.5)
70-74	7656 (28.9)	1874 (18.9)
75-79	5299 (20.0)	1871 (18.8)
80-84	3615 (13.6)	1874 (18.9)
85+	2600 (9.8)	2275 (22.9)
Race		
White	21488 (81.0)	7788 (78.4)
Black	2685 (10.1)	1347 (13.6)
Others	2359 (8.9)	798 (8.0)
Comorbidity		
Breast Cancer	1491 (5.6)	538 (5.4)
Any Cancer	3559 (13.4)	1526 (15.4)
Congestive Heart Failure	3571 (13.5)	2645 (26.6)
Ischemic Heart Disease	6715 (25.3)	3497 (35.2)
Hypertension	22456 (84.6)	8521 (85.8)
HealthCare Utilization		
Days of Hospitalization		
0	21124 (79.6)	6531 (65.8)
1 to 7	3614 (13.6)	1925 (19.4)
8+	1794 (6.8)	1477 (14.9)
N of Physician Office Visit		
≤ 3	3734 (14.1)	1782 (17.9)
4 to 6	3205 (12.1)	1083 (10.9)
7 to 12	6618 (24.9)	2245 (22.6)
13+	12975 (48.9)	4823 (48.6)

TABLE 5.1. *(Continued)* Characteristics at baseline among metformin and sulfonylureas initiators in the new user cohort

Characteristics	Metformin	Sulfonylureas
	N (%)	N (%)
Location		
Mid West	6644 (25.0)	2571 (25.9)
North East	4287 (16.2)	1898 (19.1)
South	10552 (39.8)	4113 (41.4)
West	4882 (18.4)	1279 (12.9)
Others	167 (0.6)	72 (0.7)
Prescriber Specialty		
Internal Medicine - Diabetes	586 (2.2)	180 (1.8)
Internal Medicine - Family Medicine	10213 (38.5)	3428 (34.5)
Internal Medicine - General Practice	411 (1.5)	164 (1.7)
Internal Medicine - Others	9404 (35.4)	4138 (41.7)
Physician - Others	1225 (4.6)	468 (4.7)
Physician Assistance	1964 (7.4)	550 (5.5)
Others	2729 (10.3)	1005 (10.1)
Calendar Year of Initiation		
2008	8749 (33.0)	3665 (36.9)
2009	8935 (33.7)	3389 (34.1)
2010	8848 (33.3)	2879 (29.0)

TABLE 5.2. Proportions of women receiving at least one screening test within 2-year window of 12 months before and after initiation.

Characteristics	Metformin % screened	Sulfonylureas % screened	Screening Difference (%)
Total N	49.8	33.3	16.5
Age, years			
Median	72.0	75.0	
Interquartile Range (IQR)	(69.0-77.0)	(70.0-80.0)	
Category			
65 - 69	53.9	37.5	16.4
70-74	57.5	45.4	12.1
75-79	52.2	39.6	12.6
80-84	41.6	31.5	10.1
85+	22.3	16.1	6.3
Race			
White	50.8	34.3	16.6
Black	48.2	33.2	15.0
Others	42.8	24.7	18.1
Comorbidity			
Breast Cancer	9.8	7.4	2.4
Any Cancer	35.4	27.0	8.4
Congestive Heart Failure	35.1	24.3	10.7
Ischemic Heart Disease	45.2	31.3	14.0
Hypertension	50.5	34.0	16.5
HealthCare Utilization			
Days of Hospitalization			
0	52.5	36.8	15.7
1 to 7	43.3	29.0	14.3
8+	31.5	23.7	7.8
N of Physician Office Visit			
≤ 3	29.2	16.8	12.4
4 to 6	39.8	24.3	15.5
7 to 12	51.9	32.7	19.2
13+	57.2	41.8	15.4

TABLE 5.2. ***(Continued)*** **Proportions of women receiving at least one screening test within 2-year window of 12 months before and after initiation.**

Characteristics	Metformin % screened	Sulfonylureas % screened	Screening Difference (%)
Total N	49.8	33.3	16.5
Location			
Mid West	52.2	34.8	17.4
North East	48.9	31.4	17.5
South	49.3	33.8	15.4
West	48.9	32.5	16.3
Others	40.1	19.4	20.7
Prescriber Specialty			
Internal Medicine - Diabetes	59.4	41.1	18.3
Internal Medicine - Family Medicine	48.2	31.7	16.5
Internal Medicine - General Practice	38.9	23.8	15.1
Internal Medicine - Others	52.3	34.9	17.4
Physician - Others	44.7	30.8	14.0
Physician Assistance	51.8	38.2	13.7
Others	47.9	31.2	16.6
Calendar Year of Initiation			
2008	50.0	33.0	17.0
2009	50.0	34.4	15.7
2010	49.4	32.6	16.8

TABLE 5.3. Frequency of screening mammograms and screen-detected breast cancer over 2 years in the new user cohort, by treatment group

Clinical Event	Mammograms over 24 months	
Cohort	MET	SUL
Total Patients, n	26,532	9,933
Screening Mammography		
N of screening mammograms	18,788	4,537
N of patients receiving ≥ 1 screening mammogram, n (%)	13,220 (49.8)	3,312 (33.3)
Crude RD,% (95% CI)*	16.5 (15.4 to 17.6)	
Weighted RD,% (95% CI)*,†	9.5 (8.7 to 10.3)	
Breast Cancer detected at Screening		
Screen-detected BC case, n (%)	137 (0.52)	23 (0.23)
Crude RD,% (95% CI)*	0.28 (0.16 to 0.41)	
Weighted RD,% (95% CI)*,†	0.17 (0.06 to 0.28)	
Screening detection rate for breast cancer per 1,000 tests§	7.3 (6.2 to 8.6)	5.3 (3.3 to 7.6)

Abbreviations: BC: breast cancer; N: number; MET: metformin; SUL: sulfonylureas; RD: risk difference.

* RD was estimated comparing metformin initiators to sulfonylureas initiators.

† Weighted by standardizing to their distribution in metformin initiators by using weights of 1 for metformin initiators and the odds of the estimated propensity score for sulfonylureas initiators. Propensity score model includes age, race, calendar year of initiation, and number of physician visit in 12 months prior to initiation.

§ Screening detection rate for breast cancer was calculated by total cases of screen-detected breast cancer divided by total number of screening mammograms.

TABLE 5.4. Frequency of screening mammograms and screen-detected breast cancer over 2 years in the new user cohort, stratified by time interval before and after initiation

Clinical Event	Screening mammograms before and after initiation			
	12 months before initiation		12 months after initiation	
Cohort	MET	SUL	MET	SUL
Total Patients, n	26,532	9,933	26,532	9,933
Screening Mammography				
N of screening mammograms	9,224	2,276	9,564	2,261
N of patients receiving ≥ 1 screening mammogram, n (%)	9,210 (34.7)	2,274 (22.9)	9,543 (36.0)	2,260 (22.8)
Crude RD,% (95% CI)*	11.8 (10.8 to 12.8)		13.2 (12.2 to 14.2)	
Weighted RD,% (95% CI)*,†	7.1 (6.3 to 7.8)		7.8 (7.0 to 8.6)	
Breast Cancer detected at Screening				
Screen-detected BC case, n (%)	53 (0.20)	12 (0.12)	84 (0.32)	11 (0.11)
Crude RD,% (95% CI)*	0.08 (-0.01 to 0.17)		0.21 (0.11 to 0.30)	
Weighted RD,% (95% CI)*,†	0.05 (-0.02 to 0.12)		0.12 (0.04 to 0.21)	
Screening detection rate for breast cancer per 1,000 tests§	5.9 (4.4 to 7.5)	5.7 (2.9 to 9.3)	8.9 (7.1 to 10.9)	5.3 (2.6 to 8.8)

Abbreviations: BC: breast cancer; N: number; MET: metformin; SUL: sulfonylureas; RD: risk difference.

* RD was estimated comparing metformin initiators to sulfonylureas initiators.

† Weighted by standardizing to their distribution in metformin initiators by using weights of 1 for metformin initiators and the odds of the estimated propensity score for sulfonylureas initiators. Propensity score model includes age, race, calendar year of initiation, and number of physician visit in 12 months prior to initiation.

§ Screening detection rate for breast cancer was calculated by total cases of screen-detected breast cancer divided by total number of screening mammograms.

TABLE 5.5. Characteristics at baseline among metformin and sulfonylureas initiators in the cancer-free cohort.

Characteristics	Metformin	Sulfonylureas
	N (%)	N (%)
Total N	22973 (100.0)	8407 (100.0)
Age, years		
Median	73	77
Interquartile Range (IQR)	(69-79)	(70-84)
Category		
65-69	6549 (28.5)	1821 (21.7)
70-74	6591 (28.7)	1548 (18.4)
75-79	4524 (19.7)	1578 (18.8)
80-84	3086 (13.4)	1554 (18.5)
85+	2223 (9.7)	1906 (22.7)
Race		
White	18455 (80.3)	6495 (77.3)
Black	2368 (10.3)	1188 (14.1)
Others	2150 (9.4)	724 (8.6)
Comorbidity		
Congestive Heart Failure	3028 (13.2)	2192 (26.1)
Ischemic Heart Disease	5691 (24.8)	2881 (34.3)
Hypertension	19297 (84.0)	7135 (84.9)
HealthCare Utilization		
Days of Hospitalization		
0	18633 (81.1)	5739 (68.3)
1 to 7	2914 (12.7)	1539 (18.3)
8+	1426 (6.2)	1129 (13.4)
N of Physician Office Visit		
≤ 3	3607 (15.7)	1709 (20.3)
4 to 6	3006 (13.1)	1002 (11.9)
7 to 12	5916 (25.8)	1963 (23.3)
13+	10444 (45.5)	3733 (44.4)

TABLE 5.5. (Continued) Characteristics at baseline among metformin and sulfonylureas initiators in the cancer-free cohort.

Characteristics	Metformin	Sulfonylureas
	N (%)	N (%)
Location		
Mid West	5744 (25.0)	2158 (25.7)
North East	3619 (15.8)	1565 (18.6)
South	9200 (40.0)	3519 (41.9)
West	4269 (18.6)	1104 (13.1)
Others	141 (0.6)	61 (0.7)
Prescriber Specialty		
Internal Medicine - Diabetes	467 (2.0)	147 (1.7)
Internal Medicine - Family Medicine	8906 (38.8)	2955 (35.1)
Internal Medicine - General Practice	371 (1.6)	149 (1.8)
Internal Medicine - Others	8070 (35.1)	3425 (40.7)
Physician - Others	1055 (4.6)	392 (4.7)
Physician Assistance	1749 (7.6)	475 (5.7)
Others	2355 (10.3)	864 (10.3)
Calendar Year of Initiation		
2008	7604 (33.1)	3135 (37.3)
2009	7706 (33.5)	2881 (34.3)
2010	7663 (33.4)	2391 (28.4)
Prior Screening Mammograms	8374 (36.5)	1983 (23.6)

TABLE 5.6. Proportions of women receiving at least one screening test within 2-year window of 12 months before and after initiation.

Characteristics	Metformin % screened	Sulfonylureas % screened	Screening Difference (%)
Total N	37.9	23.8	14.1
Age, years			
Median	72	74	
Interquartile Range (IQR)	(69-76)	(69-79)	--
Category			
65-69	43.2	28.8	14.4
70-74	44.1	35.5	8.6
75-79	38.9	27.1	11.8
80-84	29.7	21.3	8.4
85+	13.1	8.7	4.4
Race			
White	38.9	24.4	14.5
Black	37.7	24.3	13.4
Others	29.5	17.4	12.1
Comorbidity			
Congestive Heart Failure	24.2	15.6	8.7
Ischemic Heart Disease	32.8	21.4	11.3
Hypertension	37.8	23.7	14.1
HealthCare Utilization			
Days of Hospitalization			
0	40.1	26.9	13.2
1 to 7	32.0	18.7	13.3
8+	20.9	15.0	5.9
N of Physician Office Visit			
≤ 3	27.0	15.9	11.1
4 to 6	29.0	16.8	12.3
7 to 12	38.6	21.4	17.2
13+	43.7	30.5	13.3

TABLE 5.6. *(Continued)* Proportions of women receiving at least one screening test within 2-year window of 12 months before and after initiation.

Characteristics	Metformin % screened	Sulfonylureas % screened	Screening Difference (%)
Location			
Mid West	40.5	25.6	15.0
North East	38.0	22.2	15.8
South	37.1	24.2	12.9
West	36.2	21.7	14.4
Others	27.7	11.5	16.2
Prescriber Specialty			
Internal Medicine - Diabetes	44.1	30.6	13.5
Internal Medicine - Family Medicine	35.8	23.7	12.1
Internal Medicine - General Practice	29.1	16.8	12.3
Internal Medicine - Others	40.9	23.9	16.9
Physician - Others	33.3	22.2	11.1
Physician Assistance	40.0	28.2	11.8
Others	36.1	21.8	14.3
Calendar Year of Initiation			
2008	38.3	23.2	15.1
2009	38.1	24.5	13.6
2010	37.3	23.6	13.6
Prior Screening Mammograms	61.1	54.5	6.6

TABLE 5.7. Frequency of screening mammograms, screen-detected breast cancer, and total breast cancer over 12 months after initiation in the cancer-free cohort, by treatment group.

Clinical Event	Mammograms in 12 months after initiation	
Cohort	MET	SUL
Total Patients, n	22,973	8,407
Screening Mammography		
N of screening mammograms	8,721	1,999
N of patients receiving ≥ 1 screening mammogram, n (%)	8,701(37.9)	1,998 (23.8)
Crude RD,% (95% CI)*	14.1 (13.0 to 15.2)	
Weighted RD,% (95% CI)*, †	6.0 (5.2 to 6.9)	
Breast Cancer detected at Screening		
Screen-detected BC case, n (%)	75 (0.33)	11 (0.13)
Crude RD,% (95% CI)*	0.20 (0.09 to 0.30)	
Weighted RD,% (95% CI)* , †	0.09 (-0.01 to 0.19)	
Screening detection rate for breast cancer per 1,000 tests§	8.7 (6.9 to 10.8)	6.0 (2.9 to 10.0)
Incident Breast Cancer		
BC cases (%)	121 (0.53)	31 (0.37)
Crude RD,% (95% CI)*	0.16 (-0.00 to 0.32)	
Weighted RD,% (95% CI)*	0.06 (-0.07 to 0.19)	
% of BC detected by screening‡	62.0	35.5

Abbreviations: BC: breast cancer; N: number; MET: metformin; SUL: sulfonylureas; RD: risk difference; N/S: not specified.

* RD was estimated comparing metformin initiators to sulfonylureas initiators.

† Weighted by standardizing to their distribution in metformin initiators by using weights of 1 for metformin initiators and the odds of the estimated propensity score for sulfonylureas initiators. Propensity score model includes age, race, prior use of screening mammography, calendar year of initiation, and number of physician visit in 12 months prior to initiation.

§ Screening detection rate for breast cancer was calculated by total cases of screen-detected breast cancer divided by total number of screening mammograms.

‡ Percent of breast cancer detected by screening was calculated by cases of screen-detected breast cancer divided by total cases of breast cancers

¶ Our DUA does not allow us to present cell sizes <11, so the number for these cells were not presented on this table.

TABLE 5.8. Frequency of screening mammograms and screen-detected breast cancer over 12 months after initiation in the cancer-free cohort, stratified by receipt of screening mammography within 12 months prior to initiation.

Clinical Event	Receipt of screening mammography within 12 months prior to initiation			
	Yes		No	
Cohort	MET	SUL	MET	SUL
Total Patients, n	8,374	1,983	14,599	6,424
Screening Mammography				
N of screening mammograms	5,124	1,081	3,597	918
N of patients receiving ≥ 1 screening mammogram, n (%)	5,114 (61.1)	1,081 (54.5)	3,587 (24.6)	917 (14.3)
Crude RD,% (95% CI)*	6.6 (4.1 to 9.0)		10.3 (9.2 to 11.4)	
Weighted RD,% (95% CI)*, †	4.4 (2.8 to 5.9)		6.2 (5.2 to 7.1)	
Breast Cancer detected at Screening				
Screen-detected BC case, n (%)	29 (0.35)	<11 (<0.55)¶	46 (0.32)	<11 (<0.17) ¶
Crude RD,% (95% CI)*	N/S¶		N/S¶	
Weighted RD,% (95% CI)*, †	-0.06 (-0.26 to 0.14)		0.16 (0.05 to 0.27)	
Screening detection rate for breast cancer per 1,000 tests§	5.7 (3.8 to 8.1)	<10.2¶	12.8 (9.4 to 17.0)	<12.0¶

Abbreviations: BC: breast cancer; N: number; MET: metformin; SUL: sulfonylureas; RD: risk difference; N/S: not specified.

* RD was estimated comparing metformin initiators to sulfonylureas initiators.

† Weighted by standardizing to their distribution in metformin initiators by using weights of 1 for metformin initiators and the odds of the estimated propensity score for sulfonylureas initiators. Propensity score model includes age, race, prior use of screening mammography, calendar year of initiation, and number of physician visit in 12 months prior to initiation.

§ Screening detection rate for breast cancer was calculated by total cases of screen-detected breast cancer divided by total number of screening mammograms.

‡ Percent of breast cancer detected by screening was calculated by cases of screen-detected breast cancer divided by total cases of breast cancers

¶ Our DUA does not allow us to present cell sizes <11, so the number for these cells were not presented on this table.

TABLE 5.9. Frequency of total breast cancer over 12 months after initiation in the cancer-free cohort, stratified by receipt of screening mammography within 12 months prior to initiation.

Clinical Event	Receipt of screening mammography within 12 months prior to initiation			
	Yes		No	
Cohort	MET	SUL	MET	SUL
Incident Breast Cancer				
BC cases (%)	33 (0.39)	<11 (<0.55) ¶	88 (0.60)	21 (0.33)
Crude RD,% (95% CI)*	N/S¶		N/S¶	
Weighted RD,% (95% CI)*	-0.24 (-0.47 to -0.00)		0.21 (0.05 to 0.37)	
% of BC detected by screening‡	87.9	N/S¶	52.3	N/S¶

Abbreviations: BC: breast cancer; N: number; MET: metformin; SUL: sulfonylureas; RD: risk difference; N/S: not specified.

* RD was estimated comparing metformin initiators to sulfonylureas initiators.

† Weighted by standardizing to their distribution in metformin initiators by using weights of 1 for metformin initiators and the odds of the estimated propensity score for sulfonylureas initiators. Propensity score model includes age, race, prior use of screening mammography, calendar year of initiation, and number of physician visit in 12 months prior to initiation. For analyses stratified by receipt of prior screening mammography, propensity score was re-estimated based on the model including age, race, prior use of screening mammography, calendar year of initiation, and number of physician visit.

§ Screening detection rate for breast cancer was calculated by total cases of screen-detected breast cancer divided by total number of screening mammograms.

‡ Percent of breast cancer detected by screening was calculated by cases of screen-detected breast cancer divided by total cases of breast cancers

¶ Our DUA does not allow us to present cell sizes <11, so the number for these cells were not presented on this table.

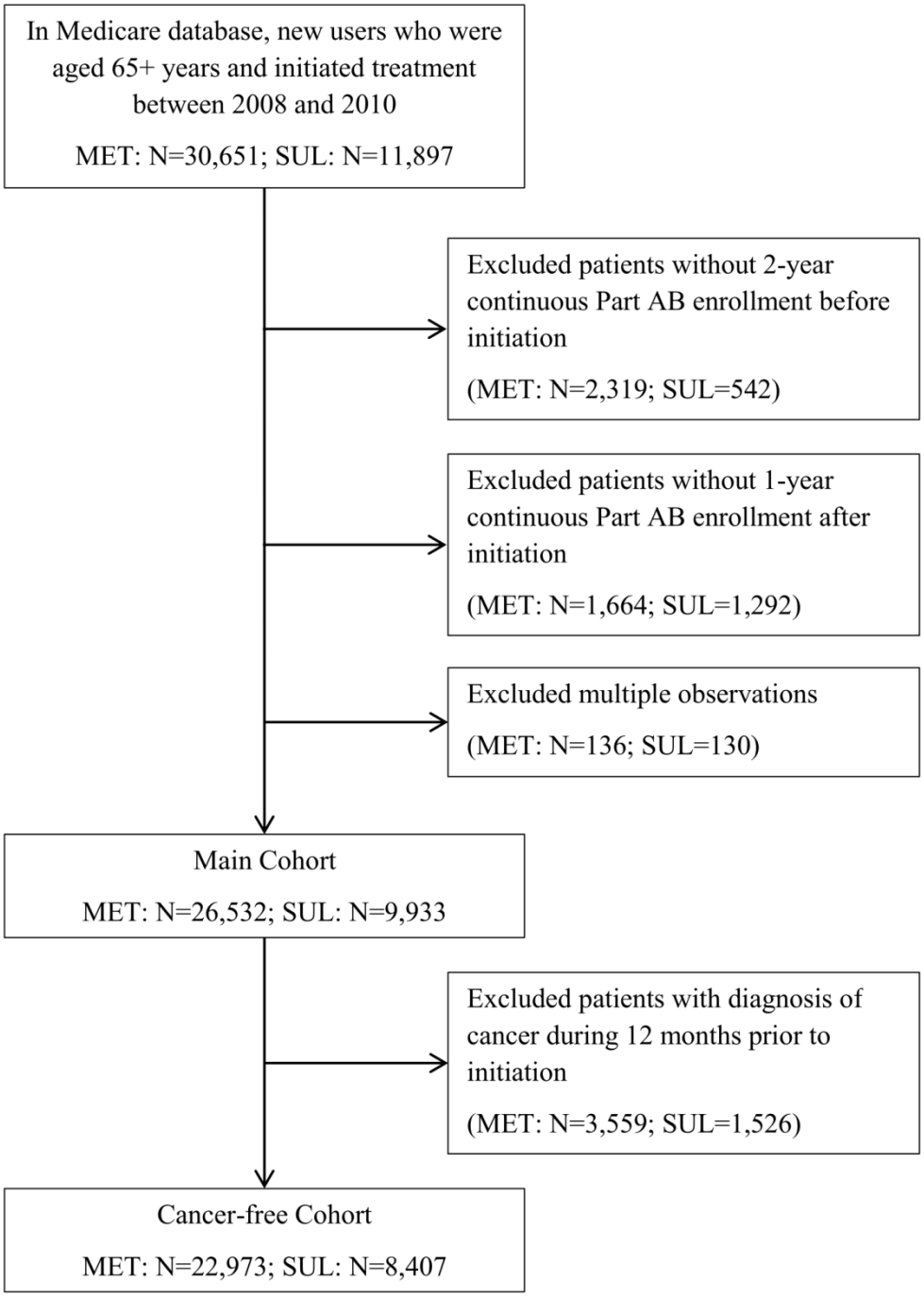


FIGURE 5.1. The flowchart of study cohort.

Abbreviations: MET: metformin; SUL: sulfonylureas.

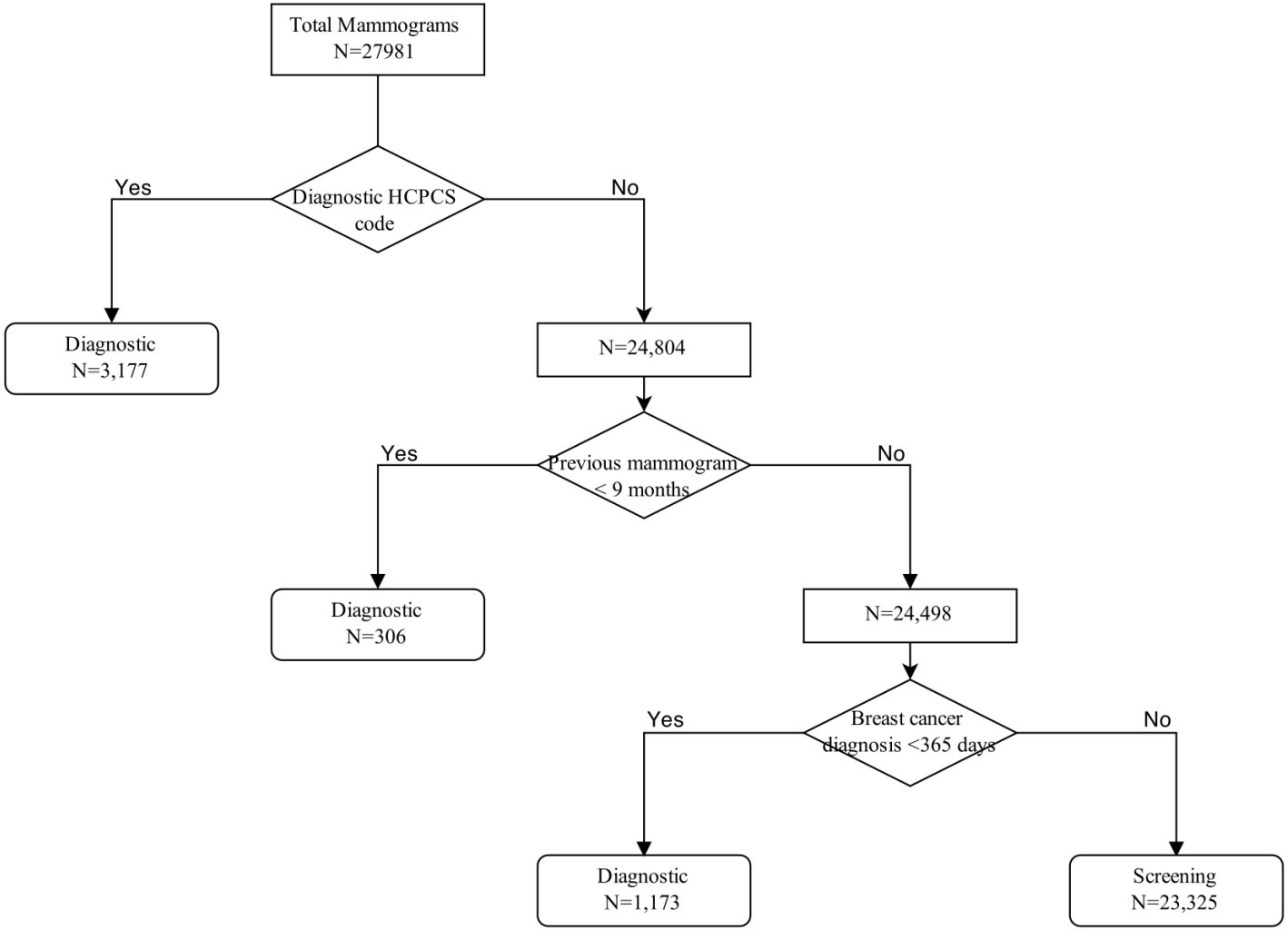


FIGURE 5.2. Allocation of mammograms by screening versus diagnostic purpose in the new user cohort.

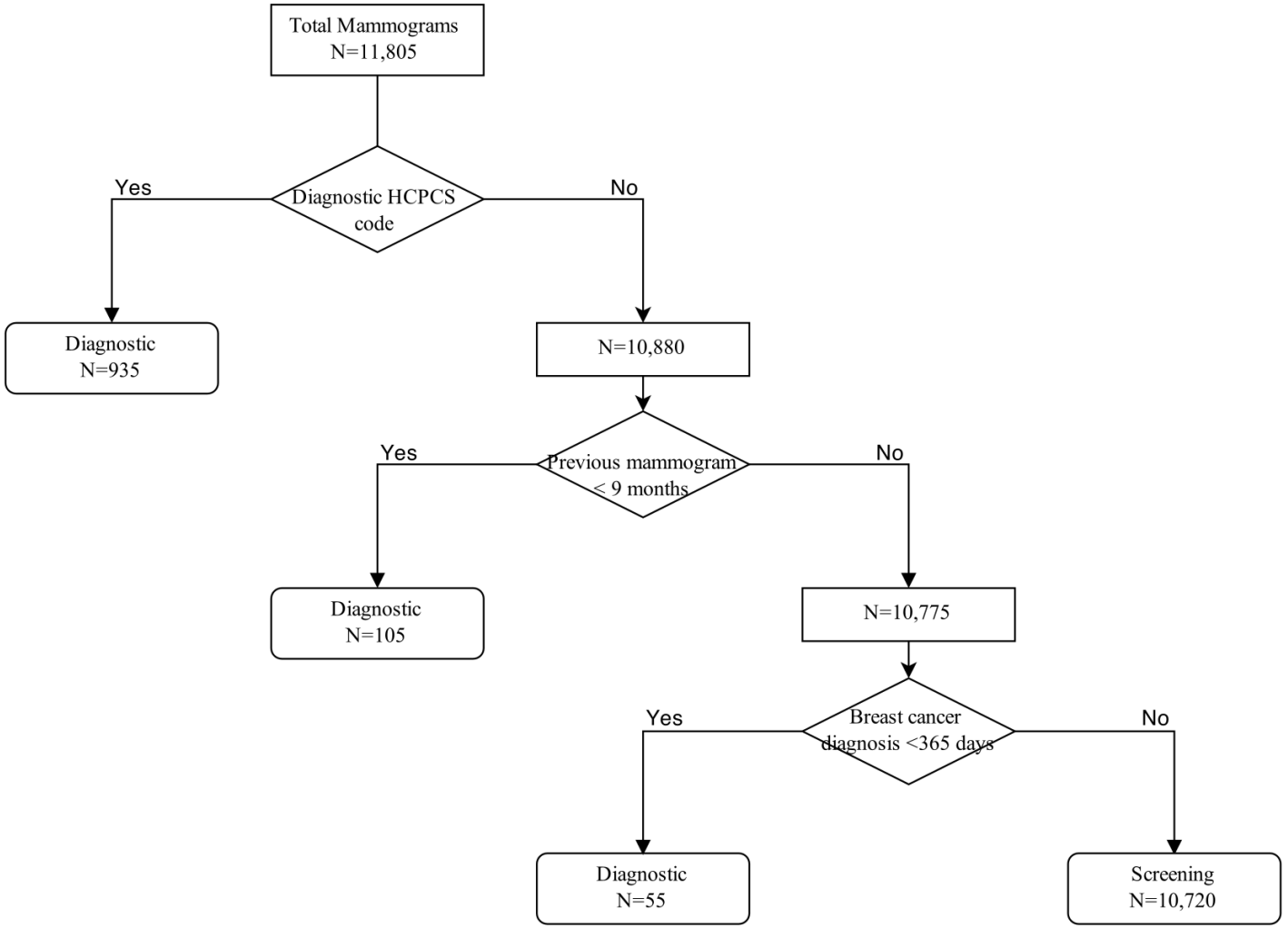


FIGURE 5.3. Allocation of mammograms by screening versus diagnostic purpose in the cancer-free cohort.

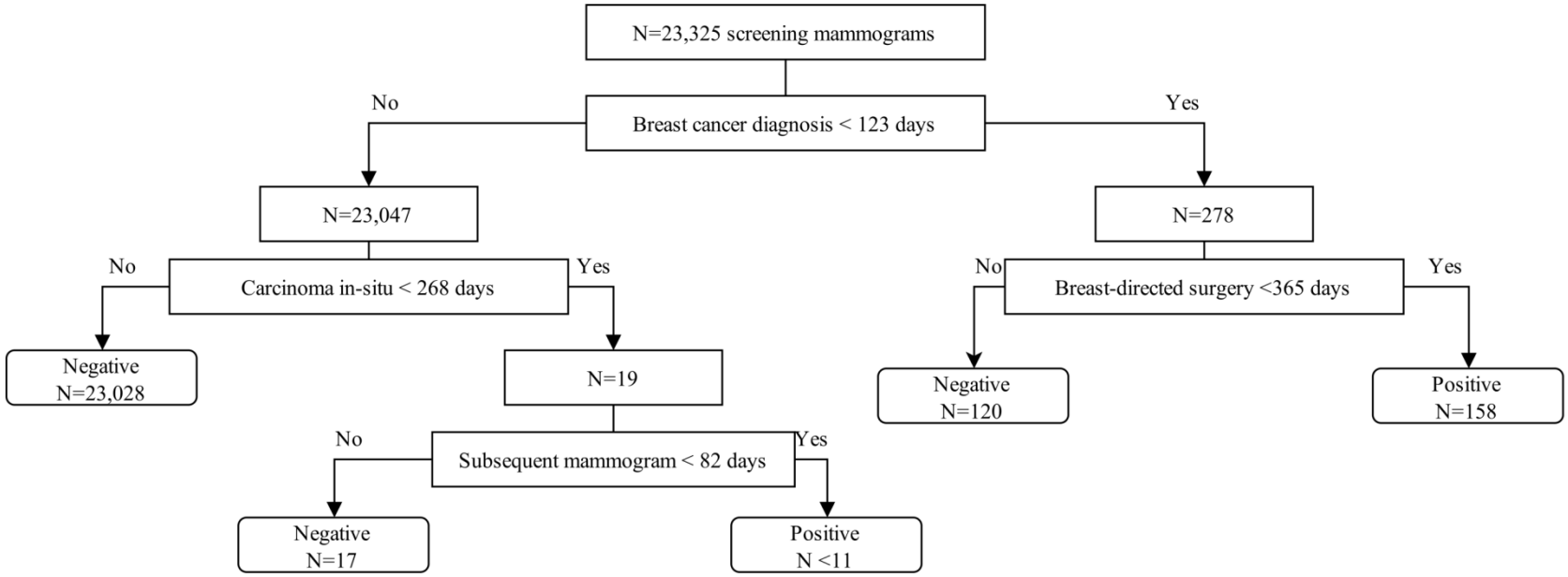


FIGURE 5.4. Algorithm for identifying incident breast cancer detected at screening mammography in the new user cohort.

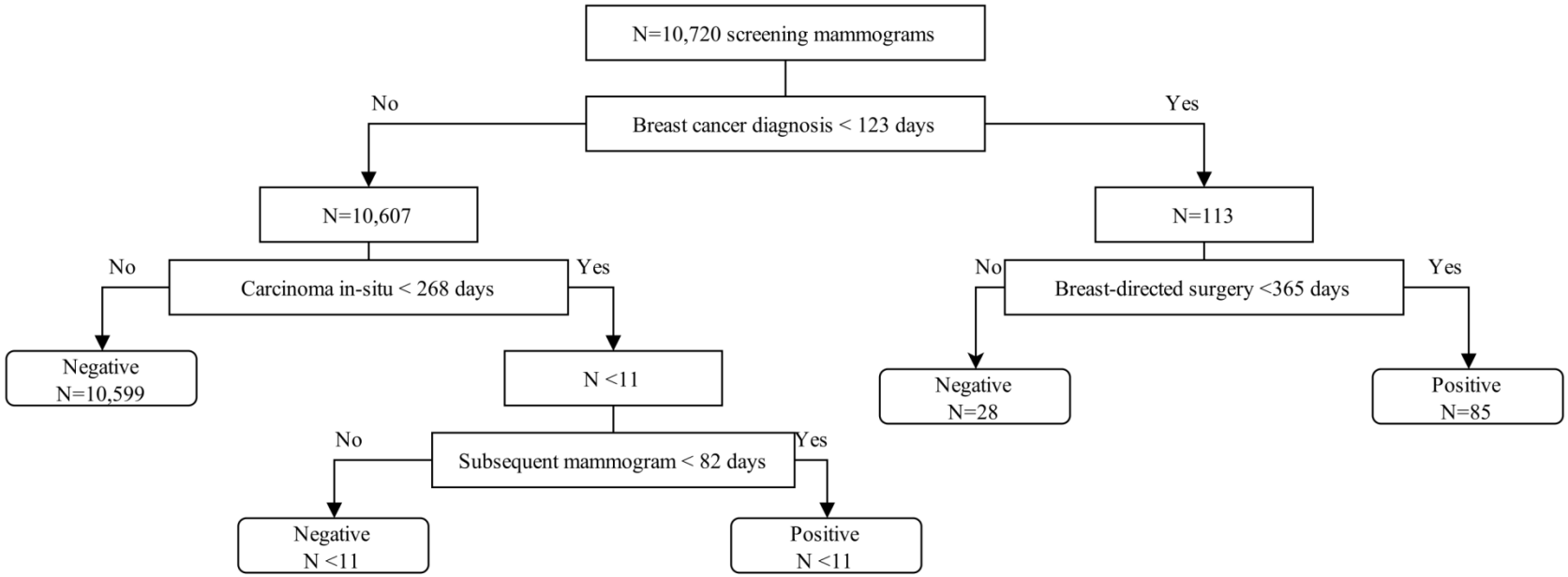
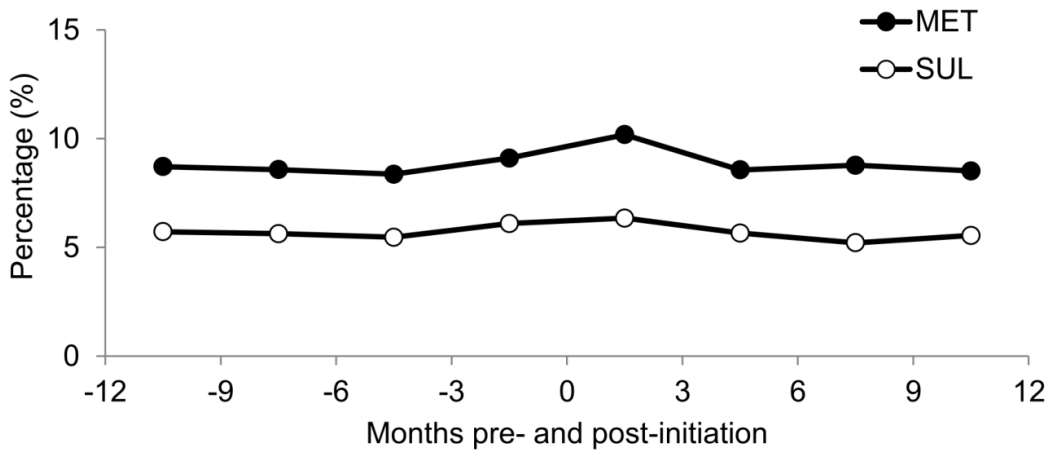


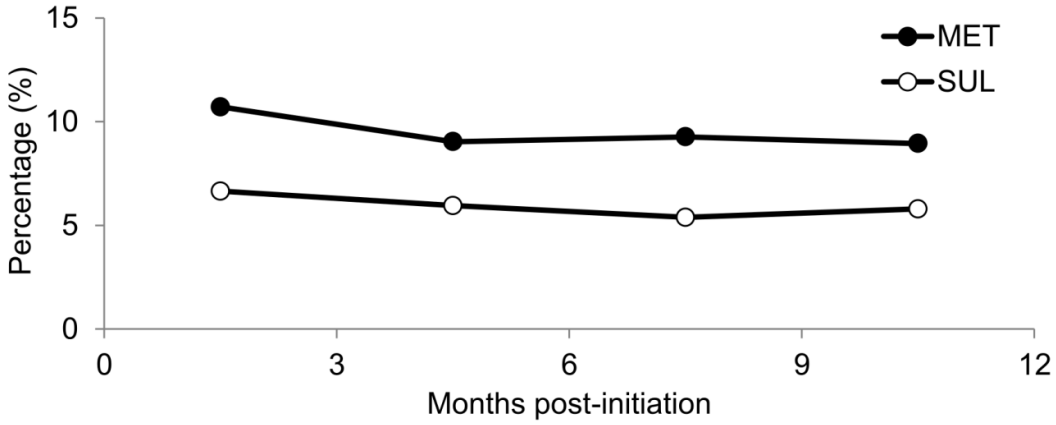
FIGURE 5.5. Algorithm for identifying incident breast cancer detected at screening mammography in the cancer-free cohort.



Proportion of patients who had screening mammograms

MET, %	8.7	8.6	8.4	9.1	10.2	8.6	8.8	8.5
SUL, %	5.7	5.6	5.5	6.1	6.3	5.7	5.2	5.5
Difference, %	3.0	2.9	2.9	3.0	3.8	2.9	3.6	3.0

FIGURE 5.6. Proportions of women receiving a screening mammogram over time by treatment group, in the new user cohort.



Proportion of patients who had screening mammograms

MET, %	10.7	9.0	9.3	8.9
SUL, %	6.6	5.9	5.4	5.8
Difference, %	4.1	3.1	3.9	3.2

FIGURE 5.7. Proportions of women receiving a screening mammogram over time by treatment group in the cancer-free cohort.

CHAPTER 6. CONCLUSIONS

I. Summary of Findings

This study used data from 2007-2011 Medicare claims to conduct the large, population-based study using a state-of-the art new user, active comparator cohort design to investigate the association between metformin and the risk of breast cancer. We found that older women initiating metformin did not have a lower risk for breast cancer than women initiating a therapeutic alternative, sulfonylureas. The results of no effects were consistent in both as-treated (AT) and intention-to-treat (ITT) analyses. The analyses stratified by duration of treatment after initiation showed no decreasing trend associated with metformin over time. No reduced risk of breast cancer associated with metformin was also found when comparing metformin initiators to initiator of Thiazolidinediones (TZD)/incretin or to diabetic initiators of angiotensin-converting-enzyme (ACE) inhibitor, and were consistent across a variety of sensitivity analyses. There was no indication of a protective effect across the age groups and in either subgroup defined by prior statin use. However, we did observe a possible tendency towards a lower risk of breast cancer associated with metformin in African American women, despite the wide corresponding confidence interval due to the small number of events.

The external validation study of Medicare Current Beneficiary Survey (MCBS) showed that being obese (BMI: ≥30) and ever smokers were not associated with metformin initiation, after multivariable adjustment, indicating little difference in BMI and smoking status conditional

on controlling for other differences. Using the information from MCBS, we used propensity score calibration (PSC) to control for unmeasured confounding by BMI and smoking in the analyses of Medicare data. We consistently observed no metformin-breast cancer associations after implementing PSC.

Receipt of screening mammography may differ between metformin and sulfonylureas initiators. This study showed that older women initiating metformin were not only more frequently screened for breast cancer in 12 months before initiation than women initiating sulfonylureas, but they also had more subsequent screening mammograms in the 12 months after initiation, especially those women not screened in the year pre-initiation. Consequently, compared with sulfonylureas initiators, metformin initiators had higher probabilities of screen-detected breast cancer both in the 12 months before and after initiation. These results indicate existence of detection bias due to differential screening mammography pre- and post-initiation when comparing breast cancer incidence between women initiating metformin and women initiating sulfonylureas.

In conclusion, our findings suggest that metformin does not reduce the risk for breast cancer among women aged 65 years or older, supporting the notion that the reduced risk of breast cancer associated with metformin observed in the previous studies is likely due to time-related biases. There was little residual confounding by unmeasured BMI and smoking status, after controlling imbalance of measured variables between metformin and sulfonylureas initiators. Despite an observation of possible detection bias due to differential screening mammography, it is unlikely explains no metformin-breast cancer association observed in this study, assuming a real protective effect of metformin. Overall, this study provides no support for

a reduced risk of breast cancer after initiation of metformin compared with a clinical alternative, sulfonylureas, in older women.

II. Strengths

This study builds on previous studies but adds to the science by reducing the potential for various biases for various reasons. First, we employed the active comparator new user design and define cohort entry and the start of follow-up carefully to avoid immortal time bias. We used the active comparator of sulfonylureas initiators in the analyses for Aim 1a (Breast Cancer Risk) and another two active comparators of TZDs/Incretins and ACE inhibitors in the sensitivity analyses. Use of active comparator group can increase comparability between cohorts and minimize the healthy user effect. Secondly, in follow-up approaches, we have given considerations on induction period (cancer development) and latency periods (carry-over effect and clinical detection for breast cancer). Cancer usually takes many years to develop and has a long latency period between a relevant exposure and the clinical detection. A disregard for the effect of latency periods might lead to a biased estimate of the association of interest. Furthermore, our study examined the potential effects of unmeasured confounders and detection bias, which has not been examined in previous metformin-breast cancer studies. The findings do no only strengthen our results from Aim 1a (Breast Cancer Risk) but also benefit future metformin-cancer studies.

III. Limitations

The study conducted to address Aim 1 (Breast Cancer and Unmeasured Variables) is limited by the short follow-up time (up to 4.5 years), and we should be aware of this limitation when interpreting the results. Nevertheless, diabetes treatment regimens are usually modified

over time for adequate glycemic control as diabetes progresses, so the observed relative short duration reflects actual treatment dynamics in the real world setting (median: 0.79 year; Interquartile Range: 0.35 to 1.65) in the AT analysis. In the ITT analysis which ignored treatment changes during follow-up, the follow-up time was almost double (median: 1.53 years; IQR: 0.69 to 2.56), but still short for evaluating a cancer outcome. We thus cannot exclude the possibility of a beneficial effect of long-term use of metformin on breast cancer risk. In addition, confounding by unmeasured variables may still exist, although we examined the impact of two major unmeasured confounders, BMI and smoking, however, suggesting little residual confounding. The Medicare and MCBS fail to capture information on some risk factors for breast cancer, such as family history of breast cancer, age at menopause and giving first birth, and drinking alcohol. We were not able to control for these variables. However, these factors are not likely to affect the choice between metformin and sulfonylureas initiation. For outcome ascertainment, we did not independently validate the diagnoses of breast cancer, but our algorithm based on ICD-9 codes has been validated with cancer registry data in an, albeit selected, Medicare population [44] and have been used extensively in pharmacoepidemiological studies.

For Aim 2 (Screening Mammography), the study is limited by the small number of screen-detected breast cancer cases, thus the effect estimates (i.e., risk difference) were imprecise. We acknowledge that we did not have a large enough study population to detect a small difference in the risk of screen-detected breast cancer during the study period. Secondly, we may have underestimated true cases of breast cancer detected at screening. To ascertain breast cancer cases, we required a breast surgery following a breast cancer diagnosis, according to the Fenton algorithm [69]. Although surgery is the primary and most effective treatment for breast cancer, it

is still possible that older and sicker women may not have surgery [77]. Given the limitations due to sample size, we did not use information on prescriber's characteristics to evaluate our speculation that differential screening test may be related to prescriber's behavior. Lastly, Medicare part B plan provides free annual screening mammography for women aged 65 years or older, reducing health inequalities for receiving mammography. Thus, our results can be generalized to US older women, but may not be generalized to younger women or women residing in other countries in which do not provide full coverage of screening mammography.

IV. Public Health Implications

This study demonstrates no beneficial effect of metformin on breast cancer risk based on the study design that minimizes the potential for bias, supporting the notion that the anti-tumor ability of metformin observed in some of the previous studies is likely due to time-related biases [7]. Randomized clinical trials have been initiated to evaluate the effect of metformin on breast cancer incidence and outcomes, and will likely be in vain, given that the observational evidence leading to these trials likely suffered from avoidable biases. Thus, our findings may provide better allocation of medical resources in the future, and emphasize the importance of conducting observational studies with rigorous, state-of-the art design to avoid spurious effects with costly consequences.

Our study is the first known study to explore the potential differential healthcare use in breast cancer screening examinations between metformin and sulfonylureas initiators. Detection bias is commonly of concern in observational studies of cancer [78, 79], including diabetes-cancer studies [38, 80, 81], but has not been examined in metformin-breast cancer studies. Our findings indicate the existence of detection bias due to differential screening mammography

between metformin and sulfonylureas initiators. Researchers should be aware of the potential for more screening mammograms pre- and post-initiation when interpreting the findings of studies assessing the effects of metformin on breast cancer incidence. Additionally, our results also point to the potential inequality of healthcare use between metformin, the first-line treatment for type 2 diabetes, and sulfonylureas, a therapeutic alternative. Although the causes are unknown, clinicians should advise and encourage diabetic patients to have cancer screening regularly.

V. Future Research

This study directs future research to several interesting topics to add to understanding of metformin's anti-tumor ability and to address some limitations of our study. First, we observed a possible tendency towards a lower risk of breast cancer associated with metformin in African American women and speculate that metformin may have distinct effect for each subtype of breast cancer, given that incidences of subtype breast cancer vary by race. Thus, future research utilizing the large databases along with detailed information of breast cancer characteristics would be desire to examine the effect of metformin on the risks of breast cancer subtypes, in particular, focusing on African American.

It is needed to identify patient and prescriber characteristics which predict prescribing metformin or sulfonylureas as initial treatment for type 2 diabetes. Recognition of these factors is informative to understand why metformin initiators were more frequently screened for breast cancer than sulfonylureas initiators, and will help clinicians and health policy makers to adequately promote screening examinations in these under-utilizing population, further improving disparity in healthcare use between metformin and sulfonylureas initiators.

Lastly, in addition to mammography, there are several common cancer screening examinations such as colonoscopy for colon cancer, prostate-specific antigen test for prostate cancer, and Pap smear for cervical cancer. And, potential anti-tumor ability of metformin has been also examined with these cancer sites. There is a need to explore potential differential use of these screening examinations between different anti-hyperglycemic drugs.

VI. Conclusions

This study provides no support for reduced risks of breast cancer after initiation of metformin compared with a clinical alternative, sulfonylureas, in older women. Our findings support the notion that reduced breast cancer risks in metformin users observed in previous studies is likely due to time-related biases, and emphasize the importance of conducting observational studies with rigorous, state-of-the art design to avoid observing spurious effects or missing real ones.

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