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Type 2 diabetes, breast cancer specific and overall mortality: associations by metformin use and modification by race, body mass, estrogen receptor, and menopausal status

By

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Master of Public Health

Epidemiology

_____ [Chair's signature]

Lauren E. McCullough, PhD, MSPH

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Pharm.D.

State University of New York at Buffalo

2010

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An abstract of
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Abstract

Type 2 diabetes, breast cancer specific and overall mortality: associations by metformin use and modification by race, body mass, estrogen receptor, and menopausal status

By Kyung Na Lee

Type 2 diabetes (T2D) has been associated with increased mortality among women diagnosed with breast cancer. While previous investigations regarding T2D and all-cause mortality have shown consistently increased mortality rates, the association between T2D and breast cancer-specific (BCS) mortality is unresolved. In addition, depending on certain prognostic characteristics─ such as race, BMI, estrogen receptor (ER), and menopausal status, heterogeneous outcomes may exist. The purpose of this paper was to examine the effect of T2D on both mortality rates and effect measure modification (EMM) by select prognostic factors. We additionally explored the association between metformin and mortality following breast cancer diagnosis. A retrospective cohort study was conducted between January 1, 2002 and December 31, 2008 from Emory University Hospitals among Non-Hispanic black and Non-Hispanic white women who had confirmed diagnosis of stage I-III invasive breast cancer and known diabetes status (T2D: n= 73; non-T2D: n= 514). Patients were followed until January 1, 2018 and were assessed for BCS and all-cause mortality. Cox proportional hazard models were used to estimate hazard ratios (HR) and corresponding 95% confidence intervals (CI). Compared to non-T2D, T2D had almost 2-fold increase in BCS mortality after adjusting for age at diagnosis, BMI, race, and comorbidities (HR=1.97; 95% CI 1.02, 3.82). Though attenuated, the increased hazard of death was also observed for all-cause mortality (HR=1.65; 95% CI 1.01, 2.69). However, interactions between T2D and mortality was not evident on multiplicative or additive scales for any of the factors considered. T2D without metformin had substantially higher HR compared to non-diabetics, a 4-fold increase in BCS mortality (HR=3.77; 95% CI 1.70, 8.36), whereas the association among T2D with metformin was modest (HR= 1.41; 95% CI 0.49, 4.03). This result may indicate a potential beneficial effect of metformin therapy on breast cancer mortality. In conclusion, among women with breast cancer T2D is associated with increased BCS and all-cause mortality. Implementing early T2D intervention program during the initial diagnosis of breast cancer may prevent excess risk of deaths.

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CHAPTER I: BACKGROUND/LITERATURE REVIEW

1.1 Breast Cancer Epidemiology

Breast cancer is the most commonly diagnosed, non-melanoma, cancer among United States (U.S.) women and the second leading cause of cancer death in the U.S. (1). It was estimated that there were about 252,710 women diagnosed with invasive breast cancer and 40,610 breast cancer deaths in 2017 (2). Gene expression profiling techniques have made it possible to understand the genetic architecture of breast cancers and molecular subtypes have been identified using biological markers such as the presence or absence of hormone receptors (estrogen and progesterone) and overexpression of the HER2 protein (1). The molecular subtypes can be categorized by immunohistochemistry makers such as ER, PR, and HER2 into Luminal A (ER+PR+/HER2-), Luminal B (ER+ PR-/HER2+), HER2-enriched (ER-PR-HER2+), and triple-negative (ER-PR-/HER2-) breast cancer types (3). Luminal A breast cancers are the most common subtype, representing 72.6% (3). While luminal A breast cancer is the most prevalent subtype among all women, the prevalence of triple-negative breast cancer (HR-/HER2-) is higher among non-Hispanic blacks than any other racial groups (3).

The incidence of breast cancer around the year 2000 started to decline after continuous increase in the past two decades, with a sharp decrease detected in between 2002 and 2003 (1). These trends were, in part, due to the Women's Health Initiative randomized trial which revealed that the use of hormone replacement therapy (HRT) associated with increased risk of breast cancer (2). Although the decrease in the use of HRT reduced overall breast cancer incidence in the U.S, female breast cancer incidence rates are known to vary by race/ethnicity. Both non-Hispanic white and black women had higher breast cancer incidence than any other racial groups, although it should be recognized that among non-Hispanic blacks that the overall trends were continuously increasing, about 0.4% per year, whereas the overall incidence rates among the non-Hispanic whites were stable (1). It was speculated that an increase in breast cancer incidence may

be due to the increase in ER+ type breast cancer, which may correlate with higher obesity rates in non-Hispanic blacks than whites (2). Breast cancer mortality rates among non-Hispanic black and white women started to diverge in the early 1980s. By 2012, the mortality rates were almost 42% higher among non-Hispanic blacks than non-Hispanic whites (1).

1.2 Risk Factors

One major cause of incident breast cancer are genetic mutations in breast cells, but only about 5% to 10% of breast cancer cases are hereditary (4). The most common genetic mutation are *BRCA1* and *BRCA2* and is typically related to early-onset breast cancer (before age 65) (5). These mutations, while highly penetrant, impact a small proportion of the population (5). Many other common, yet low penetrance genes, may also influence breast cancer; likely through their interaction with certain environmental or lifestyle factors (4). In addition to its genetic components, some proportion of breast cancer risk can be explained by non-modifiable and modifiable risk factors. Non-modifiable risk factors include age, gender, and family history (6). For instance, breast cancer incidence increases with age and doubles every 10 years until menopause. Further, women with a first-degree relative with breast cancer are at enhanced risk (5). Several other factors that are not easily modified and contribute to breast cancer risk include: reproductive factors such as age at menarche, age at first birth, parity, and age at menopause (6). Women who start menstruation before age 11, those who start their menopause after age 54, and those who gave birth to their first child in their 30s are all at higher risk of developing breast cancer (5).

Although the burden of breast cancer increases with non-modifiable risk factors (or those that are not easily modified), overall breast cancer risk can be reduced by minimizing modifiable (or lifestyle) risk factors. Weight gain is a commonly known modifiable risk factor especially among post-menopausal women, where a strong positive association between increased body mass index (BMI) and breast cancer risk has been observed (7, 8). In addition, other modifiable

risk factors include alcohol consumption, smoking, and the use of exogenous hormones (oral contraceptives/hormone replacement therapy [HRT]) (6, 9). For example, current use of HRT (compared to those who stopped HRT more than 10 years ago) was associated with higher relative risk of breast cancer (1.24; 95% CI 0.96, 1.05) (5). The collection of lifestyle factors may account for a large proportion of breast cancer cases. A study by Tamimi and colleagues showed that modifiable risk factors (*e.g.*, weight loss, no alcohol consumption, increased activity, and no HRT use) were associated with a 34.6% PAR% (Population Attributable Risk percentage) among post-menopausal women (95% CI 22.7, 45.4) (6). This result supported previous data which suggested that 26% to 40.7% of breast cancer cases arise due to lifestyle factors (6).

1.3 Obesity and Breast Cancer: Epidemiology and Mechanisms

The worldwide burden of obesity may have substantial implications for breast cancer-related morbidity and mortality (10). According to the National Health and Nutrition Examinations Survey (NHANES), about 66.2% of U.S. female adults aged 20 and over were either overweight (BMI≥25.0) or obese (BMI≥30.0), and 38.1% were obese in the year 2011-2014 (11). When stratified by race, obesity prevalence has increased from the year 1988-1994 to the year 2011-2014 among both non-Hispanic whites and non-Hispanic blacks, 22.9% to 35.3% and 38.3% to 56.5% respectively, and the prevalence of obesity has been consistently higher among non-Hispanic blacks than any other racial groups (11). A study by Arnold et al. suggested that about 2.5% (28,000 cases) of all post-menopausal breast cancer cases could have been prevented if there was no increase in BMI between 1982-2002 (10).

The positive association between obesity and breast cancer has been consistently documented among post-menopausal women. Post-menopausal women (~aged greater than 50 years) constitute more than 77% of breast cancer cases in the U.S., (6) and are at higher risk of developing invasive ER+/PR+ type breast cancer if they are overweight. The risk increases even higher for women with BMI 35.0 or greater (12). In addition to being associated with breast

cancer incidence, obesity is associated with both breast cancer-specific (BCS) and overall mortality. A meta-analysis study by Protani showed a 33% increased risk of BCS and overall mortality among obese patients (13). A more recent meta-analysis conducted by Niraula further supported this association (14), and obese post-menopausal women with ER+ breast cancer were more likely to experience recurrence and poorer BCS and overall survival (8). To this end, understanding the role of body fat in the development of breast cancer and its mechanism of action are crucial to prevent breast cancer related morbidity and mortality.

An enzyme called aromatase, found in adipose tissues as well as inside tumor tissues, is responsible for estrogen synthesis (15, 16). Estrogen levels are particularly high among obese women where, in their post-menopausal years, the primary course of endogenous estrogens are from the conversion of androgens to estrogen by aromatase in adipocytes (16). In addition, high levels of pro-inflammatory cytokines, IL6 and TNF-alpha, are associated with an increase in aromatase production and directly related to increased synthesis of estrogen (16). A nested case-control study within the Nurses' Health Study supports this relation, where the risk of post-menopausal breast cancer (particularly the ER+/PR+ subtype), was higher among women with increased levels of estrogen, and the risk of post-menopausal breast cancer increased even higher among obese women (12).

1.4 Obesity and Type 2 Diabetes

In addition to the direct association with breast cancer risk and prognosis, obesity is well-known to associate with type 2 diabetes (T2D). Despite their close association, the exact biological mechanism linking T2D to breast cancer remains unclear, although data show that women with a history of T2D are known have 15% to 20% increased risk of developing breast cancer than women without T2D (17-19). Both obesity and T2D appear to share the same pathogenesis: increased insulin resistance caused by increased pro-inflammatory cytokines (tumor necrosis factor and interleukin-6); increased inflammation and reactive oxygen species

(ROS), high insulin level and increased bioavailability of IGF-1; as well as decreased adiponectin that may either activate insulin receptor in adipose and breast tissue, or inhibit the AMP kinase (AMPK) pathway (20, 21). This shared pathophysiology of T2D and obesity can increase the odds of cancer related diseases like breast cancer by increasing cell proliferation, invasiveness, angiogenesis, and reducing the cell apoptosis (20, 21). For instance, adipose tissue is known to produce many proteins and hormones such as adipokines (22). Over-secretion of pro-inflammatory adipokines (TNF and IL-6), under-secretion of anti-inflammatory adipokines (adiponectin), and increased release of free fatty acids from adipose tissue tend to occur among obese patients (21, 23). It may affect metabolic tissues, including skeletal muscle and liver, and modify both inflammatory responses and glucose/lipid metabolism, thereby inducing metabolic syndromes like T2D (22). Both obesity and T2D have been strongly associated with oxidative stress as well, and it is speculated that an excess production of ROS can negatively activate both extracellular-related-kinase and Akt pathways to contribute to the tumorgenesis and progression (24). In addition, progressive beta-cell dysfunction observed in patients with T2D is directly linked to reduced insulin sensitivity, which can also be caused by concurrent obesity status (24). Diabetics with failed pancreas produce inadequate amount of insulin, which then leads to an excessive amount of glucose remaining in the blood and this may trigger gradual beta-cell dysfunction and insulin resistance (24). Increased insulin resistance induces inflammatory responses to release pro-inflammatory cytokines and this can cause pancreatic beta-cell death through the induction of mitochondrial stress (25). The mitochondria play an important role in cell signaling and regulating cellular metabolism to control the levels of oxygen, ATP, ROS, and the components of the apoptotic pathways. Alterations in this function can lead a metabolic changes and insulin resistance – commonly seen in both obesity and T2D (25).

1.5 Type 2 Diabetes and Breast Cancer Mortality

There have been a number of previous investigations, both meta-analytic and observational, on the association between T2D and all-cause and BCS mortality. A meta-analysis of six independent studies by Peairs and colleagues showed patients with pre-existing T2D and breast cancer had 49% higher all-cause mortality risk (HR=1.49, 95% CI 1.35, 1.65) compared to non-T2D across different populations (26). The study further showed that diabetic women presented with more advanced stage of cancer at diagnosis (26), and were more likely to experience earlier mortality. Few other observational studies supported the same conclusion of T2D as a strong predictor of increased overall mortality among breast cancer patients (18, 27-31). While numerous studies have supported strong association between T2D and all-cause mortality, studies on T2D and BCS mortality are less conclusive; with some studies indicated increased risk (27, 32), while others showed no significant differences (33-35). A meta-analysis study based on 12 studies by Zhou and colleagues indicated a 17% increased risk of BCS mortality (HR=1.17; 95% CI 1.11, 1.22) (32). A recent meta-analyses of twenty articles collected from randomized clinical trials, prospective cohort studies, and pooled cohort studies further supported increased BCS mortality rates (38% higher) among the patients with T2D compared with those without T2D (27); this result showed stronger association between T2D and breast cancer than previous studies (18, 26).

1.6 Type 2 Diabetes and Breast Cancer: Potential Effect Measure Modifiers

Given the association of T2D as a potential risk factor for breast cancer related mortality, it is of importance to consider the heterogeneous association within the strata of race, BMI, ER, and menopausal status, all of which are strong prognostic factors for breast cancer-related mortality. In a cross-sectional study of 43,701 women with incident breast cancer in the Danish breast cancer group, pre-menopausal women with T2D were more often associated with PR negative (OR=2.44; 95% CI 1.07, 5.55) and ER negative subtypes (OR=2.48; 95% CI 0.95, 6.45), which tended to have poor prognosis (36). This result was in agreement with a study by Gillespie

et al., where there was a 5-fold increase in the likelihood of developing ER negative subtypes (OR=5.22; 95% CI 1.12, 24.29) among pre-menopausal T2D patients (37), suggesting potential modification by menopausal status. In addition, the effect of T2D on breast cancer-related mortality can differ by race as well. In the study (n=9,545) by Gegechkori et al. looking at racial disparities in breast cancer mortality among the DM patients, there was a 45% increase in BCS mortality (HR=1.45; 95% CI 1.20, 1.73) among the African American (AA) after controlling for cancer-related treatment factors (38). Another study looking at the association between T2D and breast cancer subtypes among AA women indicated a 43% increased hazards of ER negative breast cancer compared to a 2% increased hazards of ER positive breast cancer, suggesting more aggressive T2D intervention may be necessary among this racial group (39). In a retrospective study of 6,342 women with breast cancer, obesity status negatively impacted survival rates, associating with an approximately 20% increased HR for BCS and all-cause mortality. This result was consistent among the DM patients where there were 12% increase in BCS mortality and 67% increase in all-cause mortality (40). Increased mortality by both obesity and DM status supported potential additive effects of two variables that could have negative impact on the overall breast cancer survival outcomes. The collective data suggest that it may be important to understand the T2D-breast cancer mortality association in the context of race, BMI, ER, and menopausal status. Additional insights would be helpful in planning T2D intervention strategies among the breast cancer patients.

1.7 Type 2 Diabetes Medications and Breast Cancer

Given that T2D is a strong risk factor for BCS and overall mortality, it is also of importance to explore the impact of diabetic medications on breast cancer. Metformin is the first line therapy to treat T2D and has been studied in many observational studies for its association with breast cancer. Despite the growing evidence of metformin's anti-cancer effect among the breast cancer patients with T2D, epidemiologic studies comparing breast cancer mortality among metformin users to non-metformin users (*e.g.*, Secretagogue, Thiazolidinedione, and Insulin)

have been inconsistent (30, 41-50), with some suggesting improved mortality rates (29, 30, 41, 42, 44-48, 51-54), while others suggesting no difference (43, 49, 50, 55).

1.8 Study Aims

Previous studies conducted on the association between T2D and mortality (both breast cancer-specific and all-cause) are inconclusive, and limitedly assessed effect measure modification by race, BMI, ER, and menopausal status. A more comprehensive understanding of the association between T2D and breast cancer, must consider these factors to develop effective interventions and treatment strategies for better control of T2D status. Therefore, this thesis aims to:

Aim 1: To examine the overall association between type 2 diabetes status and breast cancer specific mortality and overall mortality.

Aim 1a: To explore the effect of metformin vs. no metformin on breast cancer specific and overall mortality rates among patients with T2D.

Aim 2: To examine the potential heterogeneity of the association between T2D and breast cancer mortality by race, BMI, estrogen receptor (ER) status, and menopausal status.

CHAPTER II: TYPE 2 DIABETES, BREAST CANCER SPECIFIC AND OVERALL MORTALITY: ASSOCIATIONS BY METFORMIN USE AND MODIFICATION BY RACE, BODY MASS, ESTROGEN RECEPTOR, AND MENOPAUSAL STATUS

2.1 Abstract

Type 2 diabetes (T2D) has been associated with increased mortality among women diagnosed with breast cancer. While previous investigations regarding T2D and all-cause mortality have shown consistently increased mortality rates, the association between T2D and breast cancer-specific (BCS) mortality is unresolved. In addition, depending on certain prognostic characteristics─ such as race, BMI, estrogen receptor (ER), and menopausal status, heterogeneous outcomes may exist. The purpose of this paper was to examine the effect of T2D on both mortality rates and effect measure modification (EMM) by select prognostic factors. We additionally explored the association between metformin and mortality following breast cancer diagnosis. A retrospective cohort study was conducted between January 1, 2002 and December 31, 2008 from Emory University Hospitals among Non-Hispanic black and Non-Hispanic white women who had confirmed diagnosis of stage I-III invasive breast cancer and known diabetes status (T2D: n= 73; non-T2D: n= 514). Patients were followed until January 1, 2018 and were assessed for BCS and all-cause mortality. Cox proportional hazard models were used to estimate hazard ratios (HR) and corresponding 95% confidence intervals (CI). Compared to non-T2D, T2D had almost 2-fold increase in BCS mortality after adjusting for age at diagnosis, BMI, race, and comorbidities (HR=1.97; 95% CI 1.02, 3.82). Though attenuated, the increased hazard of death was also observed for all-cause mortality (HR=1.65; 95% CI 1.01, 2.69). However, interactions between T2D and mortality was not evident on multiplicative or additive scales for any of the factors considered. T2D without metformin had substantially higher HR compared to non-diabetics, a 4-fold increase in BCS mortality (HR=3.77; 95% CI 1.70, 8.36), whereas the association among T2D with metformin was modest (HR= 1.41; 95% CI 0.49, 4.03). This result may indicate a potential beneficial effect of metformin therapy on breast cancer mortality. In conclusion, among women with breast cancer T2D is associated with increased BCS and all-cause mortality. Implementing early T2D intervention program during the initial diagnosis of breast cancer may prevent excess risk of deaths.

2.2 Introduction

Breast cancer is the leading cause of cancer-related illness and the second leading cause of cancer-related death in the United States (U.S.) (1). Although the incidence of breast cancer among non-Hispanic white women is slightly higher than non-Hispanic black women (128.1 versus 124.3 cases per 100,000), non-Hispanic blacks are approximately 42% more likely to die of breast cancer than white counterparts (1).

Obesity, combined with inadequate diet and exercise, increases insulin resistance and hyperinsulinemia and is a risk factor for T2D. T2D has not only been implicated in the etiology of post-menopausal breast cancer (1-3, 7, 8), but is related to poor survival (30, 56-62) and independently associated with breast cancer cell proliferation, invasiveness, angiogenesis, and reduced cell apoptosis (20, 21, 23). Despite the body of evidence supporting the association between T2D and overall mortality (30, 56-62), conflicting results exist between T2D and breast cancer specific (BCS) mortality- with some studies supporting increased mortality rates (27, 32, 56, 62, 63), while others suggesting no increased risk of mortality (33-35). In addition, associations by important breast cancer prognostic factors (e.g., BMI, estrogen receptor [ER], and menopausal status) are unresolved (36-40), and it is suggested that the relationship between T2D and breast cancer mortality is heterogeneous by race (38, 39, 64). Given that black women are more likely to be obese (11), more likely to have ER negative subtype (11, 39), and more likely to be pre-menopausal (39), additional insight of strata specific effects could inform our understanding of racial disparities in breast cancer mortality among breast cancer patients with T2D.

Insulin resistance is the strongest biologic link between T2D and breast cancer prognosis, to date. Thus, understanding the association between T2D and breast cancer mortality in the context of diabetic medications is crucial to improving outcomes in this population (24, 41, 42). Metformin is the first-line therapy to treat T2D and has shown in numerous studies to produce anti-cancer effects through inhibition of the mammalian target of rapamycin (mTOR) pathway,

which is important in controlling cancer cell proliferations and cell growth (29, 52, 65). Although metformin's positive impact on breast cancer survival is promising, conclusive data around diabetic medications and mortality are still lacking—with some suggesting improved survival (29, 30, 41, 42, 44-48, 51-54), and others suggesting no difference (43, 49, 66, 67).

This study used a clinical database in a racially diverse sample of women treated with breast cancer in Atlanta, GA to examine the relationship between T2D and BCS and overall mortality. Atlanta is highly populated and diverse area with marked disparities in breast cancer mortality (68). We are therefore uniquely positioned to access associations considering additional factors including race, BMI, ER, and menopausal status. In addition, we will further explore the association by T2D medication use (metformin vs. no metformin) on breast cancer mortality outcomes as a sub-analysis.

2.3 Methods

Study Population

A prospective clinical database was retrospectively reviewed between January 1, 2002 and December 31, 2008, using the data obtained from de-identified medical records at Emory University Hospitals. The population of interest included all Non-Hispanic black and Non-Hispanic white women who had confirmed diagnosis of stage I-III invasive breast cancer (ICD: C509). After excluding people with unknown diabetes status, type 1 diabetes, gestational diabetes, and patients diagnosed with diabetes after breast cancer diagnosis, a total of 599 women were included in the analyses. This study was approved by the Institutional Review Board at Emory University (IRB00018512).

Exposure assessment

For our main analyses, exposure status was dichotomized as type 2 diabetics and non-type 2 diabetics. Patient's T2D status was based on their reported medical history, which was identified

and confirmed through Emory electronic medical records (EMR). The diagnostic criteria for breast cancer was based on the pathologic confirmation provided from the EMR. Among the total of 599 women diagnosed with stage I-III invasive breast cancer, T2D status was evaluated based on the prevalent clinical diagnosis made at the time of breast cancer diagnosis. 73 patients were identified to have prevalent type 2 DM, and 514 patients were without T2D after applying the exclusion criteria. Diabetic women were further divided for sub-analysis and based on their prevalent use of diabetic medications at the time of breast cancer diagnosis; 31 women were on metformin as either mono or combination therapy (metformin users), 27 women were on either sulfonylureas, thiazolidiones, or insulins as mono or combination therapy (non-metformin users), and 15 diabetic women received lifestyle modifications only (no medication users). Among the "no medication users", only 1 person experienced an event (death). As a result, this group was combined with non-metformin users for further analyses. Following the intent to treat approach, patients were divided into each group based on diabetic status and medications that they were prescribed at the time of breast cancer diagnosis.

Outcome assessment

Two separate outcomes of interest, breast cancer specific mortality and overall mortality were accessed from the Georgia Cancer Registry which has updated follow-up through November 3, 2016. Follow-up in Georgia SEER registry is considered complete through November 3, 2016 as they were matched to both State and National Death Index. However, there were 59 records with incomplete follow-up data. For those cases, EMR was used to confirm their vital status and update their date of last contact. To ensure outcome verification, patients who did not die, or those who were lost to follow-up were censored at their last date of follow-up. Completed follow-up data from Georgia SEER registry through November 3, 2016 was then compared with EMR again and updated patient vital status if they were seen again after November 3, 2016 through January 1, 2018, which was the final censoring date for this study. BCS mortality was defined as

the time until the death due to the breast cancer, identified from the cancer registry (ICD: C509) (69). Overall mortality was defined as the time until death from any cause (including, but not limited to breast cancer).

Covariate assessment

Age at breast cancer diagnosis (continuous variable), race (non-Hispanic white vs. non-Hispanic black), and BMI (kg/m^2) according to WHO definitions (non-obese BMI<30 and obese BMI≥30) were accessed as potential confounders. For those patients whose BMI was not available at the time of breast cancer diagnosis, it was obtained during the 12 months post diagnostic period from the EMR. Menopausal status (pre-menopausal vs. post-menopausal), medication use (metformin vs. no metformin vs. no medications), and comorbidities (hypertension, heart disease, hypercholesterolemia, and other disease) were determined as part of the covariate assessments as well. Breast cancer clinical stage according to American Joint Committee on Cancer was divided into stages I, II, and III based on the accessibility of primary tumor (T), regional lymph nodes (N), and distant metastases (M) (70). Tumor grade was categorized as stages I/II, and III based on the abnormality of the tumor cells. Tumor characteristics based on the patient's ER status (positive vs. negative) was abstracted from the Emory EMR. Lastly, the receipt of standard treatments (surgery, chemotherapy, radiation) were also evaluated as part of the covariate assessments. Among all potential covariates, confounding assessment was performed using directed acyclic graph (DAG) analyses (71). For our main analysis and sub-analysis, both clinical and treatment characteristics were removed from the model as they were in the causal pathways between T2D and mortality, violating our confounding criteria. Menopausal status was dropped from the model as well because there was less than 10% change in estimate when included compared to the fully adjusted model which included age at diagnosis, race, BMI, menopausal status, and comorbidities.

Statistical Analysis

Patient demographics and their clinical and treatment characteristics were compared between groups using chi-square test and fisher's exact test. The association between T2D and breast cancer mortality outcomes was evaluated by generating multi-variate adjusted survival curves. Survival curves based on the main exposure followed by T2D medications, as well as the effects stratified by race, BMI, ER, and menopausal status were also compared. A time to event analyses were performed by setting the date of breast cancer diagnosis as the index date. For the main effect, the multivariate Cox regression analysis (71) was performed to evaluate the effect of T2D on breast cancer mortality outcomes after adjusting for age at diagnosis, race, BMI, and comorbidities. Potential modification of the association between T2D and breast cancer mortality was evaluated by race, BMI, ER, and menopausal status using the likelihood ratio test (LRT) to assess interaction on the multiplicative scale as well as the interaction contrast ratio test (ICR) to evaluate the interaction on the additive scale (72). In addition, the effect of diabetic medications (metformin vs. no metformin) on mortality was explored using the Cox proportional hazards regression after adjusting for the same covariates. We performed visual assessment of the proportional hazards assumption in addition to test of goodness of fit, and time-dependent covariate method. Upon testing each covariate for the proportional hazards assumption, no violations were observed. All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC).

2.4 Results

Patient demographics and clinical/treatment characteristics

Table 1 represents patient demographics and clinical/treatment characteristics, categorized by patient's T2D status. In comparison to non-T2D, T2D women were older, Non-Hispanic blacks, obese, post-menopausal at breast cancer diagnosis, and presented with more comorbid conditions.

With the exception of tumor grade, the two groups were similar with respect to clinical and treatment characteristics.

BCS and overall mortality rates by T2D status and diabetic medications

With a mean follow-up times of 93.82 months among T2D women, 26 (40.0 %) deaths occurred, 14 (21.5%) due to breast cancer. In the non-T2D group, the mean follow-up time was 115.38 months. During this time 102 (21.1 %) deaths occurred, in which 64 (13.2 %) deaths were due to breast cancer. Figure 1 shows multi-variate adjusted survival curves for breast cancer specific survival (BCSS) (Figure 1a) and overall survival (OS) (Figure 1b) between T2D and non-T2D. Compared to non-T2D, T2D women had a 2-fold increase in BCS mortality (HR=1.97; 95% CI 1.02, 3.82) after adjusting for age at diagnosis, BMI, race, and comorbidities (Table 2). The risk of death attenuated with overall mortality (HR=1.65; 95% CI 1.01, 2.69) after adjusting for the same covariates. When we assessed these associations in the strata of medication use, we observed that for both BCSS and OS, metformin users had improved survival compared to T2D women without metformin (Figure 2). Similarly, in Table 2 we showed that there was a monotonic increase in the risk of BCS deaths among the women with T2D according to medication use. The risk among metformin users was slightly higher than non-T2D (HR=1.41; 95% CI 0.49, 4.03), but women with T2D without metformin had the highest risk of BCS mortality (HR=3.77; 95% CI 1.70, 8.36). Similar trends were observed for overall mortality where slightly higher death rate was shown among the metformin users compared to the non-T2D (HR=1.53; 95% CI 0.77, 3.05), but non-metformin users had almost 2.5-fold increase in deaths compared to the non-T2D (HR=2.69; 95% CI 1.40, 5.18).

BCS and overall mortality rates by T2D status within the strata of effect measure modifiers

Race

Within the strata of race, stratified HRs among non-Hispanic whites were slightly higher for T2D than non-T2D (HR=1.25; 95% CI 0.37, 4.21) and we observed a near 3-fold increase in BCS mortality among non-Hispanic blacks with T2D compared to the non-T2D (HR=2.98; 95% CI 1.24, 7.18) (Table 3). Using single referent coding to estimate the joint probability of risk, we found that non-Hispanic blacks with T2D had the worst BCS mortality rates (HR=2.79; 95% CI 1.16, 6.72). Similar trends were exhibited for overall mortality rates. We found no significant interactions by race on either multiplicative or additive scales.

BMI

Among the obese women with T2D we observed a greater than 3-fold increase in BCS deaths compared to non-T2D obese women (HR=3.12; 95% CI 1.33, 7.32, Table 3). In the combined effect, we similarly found that obese women with T2D were most likely to experience BCS mortality (HR=4.33; 95% CI 1.97, 9.50), followed by non-T2D obese women (HR=1.39; 95% CI 0.74, 2.59), although interactions on multiplicative or additive scales were not apparent. Though attenuated, these associations were also present for overall mortality rates.

ER Status

Within the strata of ER status (Table 3), T2D case women with ER positive breast cancer experienced an 85% increased risk of death compared to non-T2D (HR=1.85; 95% CI 0.67, 5.08). Among ER negative breast cancer cases with T2D, the HR was 2.36 (95% CI 0.93, 6.01), yet precise. When considering the estimated HR for the joint effect of T2D and ER status, a 6-fold increase in hazard was observed among the women with T2D with ER negative breast cancer (HR=6.08; 95% CI 2.35, 15.73). For overall mortality, similar patterns were observed. Interactions between T2D and ER status were not present on multiplicative or additive scales for either outcome.

Menopausal Status

Within the strata of menopausal status (Table 3), the association of T2D on BCS mortality was apparent among the post (HR=1.89; 95% CI 0.86, 4.16) and pre-menopausal (HR=3.33; 95% CI

0.75, 14.84) T2D women compared to non-T2D women. Using the common referent coding we observed that pre-menopausal women with T2D were at the highest risk for BCS and overall mortality (HR=3.30; 95% CI 0.66, 16.54 and HR=5.52; 95% CI 1.21, 25.27 respectively). However, interactions were not present on multiplicative or additive scales.

2.5 Discussion

In our study, breast cancer patients with T2D had 97% increased risk of BCS mortality and 65% increased all-cause mortality compared to the breast cancer patients without T2D. Our findings may suggest that having T2D is a strong prognostic factor for breast cancer-related deaths. Although we did not observe interaction between T2D and race, BMI, ER, or menopausal status on multiplicative or additive scales, assessing EMM by these factors may be important for targeting future interventions among T2D women diagnosed with breast cancer. Finally, our sub-analysis on BCS mortality rates among metformin users were substantially lower than that of T2D women without metformin, suggesting metformin may be a therapeutic agent for this subset of women.

The positive association between T2D status and overall mortality observed in our study was consistent with meta-analysis where a 37% increased risk of all-cause mortality was observed (HR=1.37; 95% CI 1.34, 1.41) (32). This result was in agreement with other observational studies as well (18, 27-31, 63). However, previous studies on the association between T2D and BCS mortality rates were mixed. Two prospective studies conducted by Yeh et al., and Chen et al. (63, 73) (n=18,280 and n=4,390 respectively) showed 27% and 53% increased BCS mortality (95% CI 0.17, 9.73 and 95% CI 1.14, 2.05 respectively), which was further supported by a retrospective cohort study among Swedish women diagnosed with breast cancer (n= 146,764) where the hazard of death was 1.45 (95% CI 1.32, 1.59) (62). In contrast, two retrospective studies with relatively large sample size showed no associations between T2D status and BCS mortality rates (33, 35). In a prospective cohort (n=4,664) by Nechuta et al.,

investigators identified no significant association (HR=0.98; 95% CI 0.68, 1.41) (35), consistent with finding from Luo et al. (n=8,108) after accounting for competing risks of death (HR=0.90; 95% CI 0.65, 1.24) (33). Despite the conflicting results, there is a general consensus that prevalent T2D at the time of breast cancer diagnosis is clearly associated with mortality among the breast cancer patients (27, 32, 62-65, 56, 73). Our result of 97% increase in BCS mortality is consistent with this, although, given a relatively small sample size and small number of patients who experienced BCS deaths (n=14), should be interpreted with caution.

In our study, we additionally examined EMM to estimate the effect of T2D status within different covariates of interest (71). Although EMM on two different scales were not statistically significant, additive interaction was still in the positive direction, where the combined effect was larger than the sum of the individual effects, indicating the potential benefit of T2D intervention among select racial, BMI, ER, and menopausal groups. T2D intervention among pre-menopause is important to consider for BCS survival given that pre-menopausal women with T2D tend to present with ER negative subtype that is more aggressive to treat and has poor prognosis (36, 37, 74). In addition, a previous study has shown increased risk of ER negative breast cancer among non-Hispanic blacks with T2D (39), suggesting that T2D control in this group may narrow mortality disparities. Finally, it has been suggested that obese patients with T2D may have delayed detection due to increased breast fat tissue, altered biological mechanisms due to macrophage infiltration, more aggressive breast tumors, and increased odds of comorbid conditions (40, 75). While our data were suggestive, larger samples with incident T2D cases are needed to make strong conclusions on these potential interactions.

Increased risk of deaths among T2D with breast cancer is thought to be both directly and indirectly related to hormonal, inflammatory, or metabolic characteristics of T2D (32). T2D, often combined with obesity, induces hormonal changes by increasing insulin resistance, inflammation with increased Interleukin 6, and increased reactive oxygen species, all of which can lead to hyperinsulinemia and insulin-stimulated mitosis by directly affecting cancer cell

proliferation (22). Increased insulin level can also indirectly stimulate tumor cell growth by increasing the bioavailability of insulin-like-growth-factor-1 (IGF-I) through glucogenesis, which can activate insulin receptors in various tissues including breast cells through stimulation of abnormal signaling cascade that can increase angiogenesis and reduced apoptosis (22, 23). In addition, hyperinsulinemia can decrease sex-hormone-binding globulin that can increase estrogen concentration and enhance estrogen receptor activation through IGF-I signaling (22). It is known that a synergistic effect of estrogen and IGF-I can lead to breast cancer cell proliferation among diabetic patients (23).

From the biological association and different mechanisms involved with T2D and breast cancer mortality, diabetic medications such as metformin may have a favorable effect on survival among the breast cancer patients. Hyperinsulinemia has been strongly associated with increased breast cancer risk, and metformin is known to provide both direct and indirect effect in reducing cell proliferations through controlling insulin levels and blood glucose (65). Metformin indirectly activates adenosine monophosphate-activated protein kinas (AMPK), which then inhibit the mammalian target of rapamycin (mTOR) to prevent breast cancer cell proliferation as well as to stop cell growth and pathological cell cycle progression (65). Previous studies on the effect of metformin on breast cancer mortality is mixed although growing evidence suggests potential effect as an anti-cancer agent. Retrospective clinical meta-analysis were performed on 28 studies on metformin use and showed an inverse association with the pooled effect estimate of 0.70 (95% CI 0.55, 0.88), a decrease in the risk of all-cause mortality among breast cancer patients with concurrent DM (76). Although our study result was based on relatively small number of events, and effects imprecise, the observed hazard of death was considerably lower among the metformin users vs. non-T2D than the non-metformin users vs. non-T2D, which may suggest a protective effect of metformin therapy on survival outcomes. A large population-based study (n=1,058) taking place in Denmark also supported this premise. These data showed reduction in both BCS and all-cause mortality rates with cumulative metformin use (HR=0.74; 95% CI 0.58, 0.96 and

HR=0.88; 95% CI 0.59, 1.29 respectively) (48). This finding was consistent with the results of another population-based study (n=2,361) by Lega et al., where there was a non-significant 9% reduction in BCS mortality for cumulative metformin use (50). However, metformin's protective effect needs to be interpreted with caution as above study results could be slightly underestimated due to the confounding by indication (48, 71). T2D status confounds metformin to mortality outcome pathway since breast cancer patients with T2D are likely to receive metformin due to its common use as a first line drug therapy for T2D. In addition, the severity of T2D is likely to increase the mortality outcomes, creating a confounding pathway. Unless instrumental variable as a proxy for metformin is used to control this pathway, the overall effect of metformin on mortality outcome can be confounded by T2D status and may bias the results towards more protective effect (48, 71).

The strengths of our study include its use of large cohort of women diagnosed with stage I to III invasive breast cancer. We were also able to access EMM on both multiplicative and additive scales as compared to previous studies that have not extensively looked at departures from additive effects. Accessing additive interaction may be more relevant for public health relevance and biological interaction (71). The limitation of our study is that we were not able to confirm laboratory measurement of T2D status and it was based only on the prevalent T2D status at the time of breast cancer diagnosis, which could have possibly biased our result. In addition, those patients whose T2D status was missing at the time of breast cancer diagnosis were excluded from our study and may bias our estimated associations. We also did not take into the consideration of the severity of T2D, which may lead to the overestimation of our BCS and overall mortality rates. In addition, no prior information was provided on the duration of therapy or treatment regimen which may affect the disease progression. Treatment therapy could change over time from one medication to another, and we did not account for time varying exposure. Instead of dichotomizing metformin status as metformin vs. non-metformin, treating metformin as time-dependent covariate could avoid immortal time bias (77), since those women who were

initially unexposed to metformin but later became exposed will be counted as “unexposed” and it will not affect the personal time of women who were initially exposed to metformin (77). Lastly, sample size of women with T2D and those who experienced an event were relatively small and came from a highly selected pool of patients. Thus, we were underpowered to detect differences by key covariates on both multiplicative and additive scales.

In conclusion, our findings show that prevalent T2D status at the time of breast cancer diagnosis can increase BCS mortality and all-cause mortality among the women diagnosed with stage I-III invasive breast cancer. Further study is needed to investigate the causal relationship between the incident T2D cases and breast cancer in clinical settings, particularly in subgroups of race, BMI, ER, and menopausal status, where targeted intervention (such as metformin) may prevent and minimize excess risk of deaths among women diagnosed with breast cancer.

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2.7 TABLES

Table 1. Patient demographics and clinical/treatment characteristics by type 2 diabetes status among the patients with Breast Cancer at diagnosis

Characteristics	Type 2 Diabetics (N=73)	Non-Type 2 Diabetics (N=514)	P-value
Age at diagnosis, years			
Median (range)	62.00 (32 - 82)	52.50 (22 - 89)	<0.0001
	No. (%)	No. (%)	
Race			<0.0001
Non-Hispanic white	27 (36.99)	330 (64.20)	
Non-Hispanic black	46 (63.01)	184 (35.80)	
Missings	0	0	
BMI (kg/m2)[1]			0.0003
< 30 (non-obese)	41 (56.16)	360 (70.04)	
≥ 30 (obese)	28 (38.36)	125 (24.32)	
Missings	4 (5.48)	29 (5.64)	
Menopausal status			<0.0001
Pre-menopausal	5 (6.85)	194 (37.74)	
Post-menopausal	65 (89.04)	300 (58.37)	
Missings	3 (4.11)	20 (3.89)	
Medications			<0.0001
Metformin	31 (42.47)	0 (0.00)	
No Metformin[2]	27 (36.99)	0 (0.00)	
No medications	15 (20.55)	512 (99.61)	
Missings	0 (0.00)	2 (0.39)	
Comorbidities			<0.0001
No	7 (9.59)	162 (31.52)	
Yes	66 (90.41)	348 (67.70)	
Missings	0 (0.00)	4 (0.78)	
Hypertension	53 (72.60)	180 (35.02)	
Heart Disease[3]	17 (23.29)	40 (7.78)	
Hypercholestrelomia	27 (36.99)	66 (12.84)	
Others	10 (13.70)	180 (35.02)	

Table 1. Continued…

Characteristics	Type 2 Diabetics (N=73)	Non-Type 2 Diabetics (N=514)	P-value
Clinical Characteristics			
Clinical stage			0.10
I	28 (38.36)	198 (38.52)	
II	22 (30.14)	209 (40.66)	
III	23 (31.51)	107 (20.82)	
Missings	0 (0.00)	0 (0.00)	
Tumor grade			0.03
I and II	33 (45.21)	283 (55.06)	
III	33 (45.21)	182 (35.41)	
Missings	7 (9.59)	49 (9.53)	
ER Status			0.14
positive	46 (63.01)	342 (66.54)	
negative	23 (31.51)	162 (31.52)	
Missings	4 (5.48)	10 (1.95)	
Treatment characteristics			
Surgery			0.16
Partial Mastectomy	9 (12.33)	116 (22.57)	
Total Mastectomy	27 (36.99)	153 (29.77)	
Missings	37 (50.68)	245 (47.67)	
Chemotherapy			0.74
No	17 (23.29)	99 (19.26)	
Yes	44 (60.27)	309 (60.12)	
Missings	12 (16.44)	106 (20.62)	
Radiation			0.63
No	4 (5.48)	50 (9.73)	
Yes	61 (83.56)	424 (82.49)	
Missings	8 (10.96)	40 (7.78)	

[1] BMI: Body Mass Index

[2] Includes Sulfonylureas, Thiazolidiones, or Insulins as monotherapy or as combination therapy

[3] Includes arterial fibrillation, arrhythmia, Coronary Artery Disease, Cardiovascular disease, Myocardial Infarction, and Mitral Valve prolapse

Table 2. Multivariate Cox Proportional Hazard Model showing the association between type 2 diabetes (T2D) vs. non-type 2 diabetes (non-T2D) on Breast Cancer-Specific (BCS) and Overall mortality as well as the effects by different types of T2D medications (metformin vs. no metformin vs. non-T2D)

	Breast Cancer Specific (BCS) Mortality			Overall Mortality		
	events/p-month[1]	Hazard Ratio (HR)[a]	95% CI	events/p-month[1]	Hazard Ratio (HR)[a]	95% CI
Main effect						
non-T2D	64/55,960	1.00	ref	102/55,960	1.00	ref
T2D	14/6,098	1.97	1.02 to 3.82	26/6,098	1.65	1.01 to 2.69
Sub-Analysis						
Diabetes Medications						
non-T2D	64/55,960	1.00	ref	102/55,960	1.00	ref
T2D with metformin	5/3,101	1.41	0.49 to 4.03	11/3,101	1.53	0.77 to 3.05
T2D with no metformin	9/1,672	3.77	1.70 to 8.36	14/1,672	2.69	1.40 to 5.18

[1] events/p-month: number of death occurred by the sum of total time in month contributed by all patients
[a] Adjusted for age at diagnosis, BMI, race, and comorbidities

Table 3. Multivariate Cox Proportional Hazard Model showing the association between T2D vs. non-T2D on mortality outcomes by the strata of race, BMI and ER, and menopausal status (EMM) on multiplicative scale (stratified HR) and the combined effect of T2D and EMM on additive scale (single referent HR) for both BCS and overall mortality

	Breast Cancer Specific (BCS) Mortality					Overall Mortality				
		Stratified HR[3]		Single referent HR[4]			Stratified HR[3]		Single referent HR[4]	
	event/ P-month[2]	HR (95% CI)	LRT P-value	HR (95% CI)	Additive ICR/ RERI (95% CI)	event/ P-month[2]	HR (95% CI)	LRT P-value	HR (95% CI)	Additive ICR/ RERI (95% CI)
Race			0.23					0.13		
Non-Hispanic White										
non-T2D	36/ 37,099	1.00		1.00		53/ 37,099	1.00		1.00	
T2D	3/2,836	1.25 (0.37 to 4.21)		1.25 (0.37 to 4.21)		5/2,836	1.02 (0.40 to 2.65)		1.02 (0.40 to 2.65)	
Non-Hispanic Black										
non-T2D	28/ 18,861	1.00		0.94 (0.50 to 1.74)		49/ 18,861	1.00		1.38 (0.85 to 2.24)	
T2D	11/3,262	2.98 (1.24 to 7.18)		2.79 (1.16 to 6.72)	1.60 (-0.83 to 4.03)	21/ 3,262	2.35 (1.23 to 4.48)		3.01 (1.54 to 5.88)	1.84 (-0.36 to 4.04)
BMI (kg/m2)[1]			0.13					0.34		
< 30 (non-obese)										
non-T2D	41/ 40,417	1.00		1.00		65/ 40,417	1.00		1.00	
T2D	4/3,276	0.95 (0.22 to 4.07)		0.95 (0.22 to 4.07)		11/ 3,276	1.31 (0.58 to 2.99)		1.31 (0.58 to 2.99)	
≥ 30 (obese)										
non-T2D	16/ 13,338	1.00		1.39 (0.74 to 2.59)		27/ 13,338	1.00		1.34 (0.82 to 2.19)	
T2D	9/2,565	3.12 (1.33 to 7.32)		4.33 (1.97 to 9.50)	2.99 (-0.63 to 6.61)	13/2,565	2.17 (1.09 to 4.32)		2.90 (1.53 to 5.51)	1.25 (-0.71 to 3.21)
ER Status			0.72					0.82		
ER positive										
non-T2D	31/ 38,223	1.00		1.00		53/ 38,223	1.00		1.00	

Table 3. Continued…

	Breast Cancer Specific (BCS) Mortality					Overall Mortality				
		Stratified HR[3]		Single referent HR[4]			Stratified HR[3]		Single referent HR[4]	
	event/ P-month[2]	HR (95% CI)	LRT P-value	HR (95% CI)	Additive ICR/ RERI (95% CI)	event/ P-month[2]	HR (95% CI)	LRT P-value	HR (95% CI)	Additive ICR/ RERI (95% CI)
T2D	5/4,151	1.85 (0.67 to 5.08)		1.85 (0.67 to 5.08)		14/4,151	1.66 (0.86 to 3.20)		1.66 (0.86 to 3.20)	
ER negative										
non-T2D	30/ 17,024	1.00		2.57 (1.47 to 4.51)		45/ 17,024	1.00		1.97 (1.27 to 3.06)	
T2D	7/1,796	2.36 (0.93 to 6.01)		6.08 (2.35 to 15.73)	2.66 (-0.62 to 5.94)	9/1,796	1.88 (0.80 to 4.38)		3.69 (1.60 to 8.53)	1.06 (-0.56 to 2.69)
Menopausal Status			0.44					0.30		
post-menopause										
non-T2D	37/ 31,543	1.00		1.00		70/ 31,543	1.00		1.00	
T2D	12/ 5,262	1.89 (0.86 to 4.16)		1.89 (0.86 to 4.16)		23/ 5,262	1.59 (0.91 to 2.79)		1.59 (0.91 to 2.79)	
pre-menopause										
non-T2D	24/ 22,430	1.00		0.99 (0.45 to 2.17)		27/ 22,430	1.00		1.39 (0.73 to 2.66)	
T2D	2/531	3.33 (0.75 to 14.84)		3.30 (0.66 to 16.54)	1.41 (-3.55 to 6.38)	2/531	3.97 (0.92 to 17.19)		5.52 (1.21 to 25.27)	3.54 (-4.63 to 11.71)

[1] BMI: Body Mass Index

[2] events/P-month: number of death occurred by the sum of total time in month contributed by all patients

[3] The effect of T2D on survival was stratified by 4 effect measure modifiers (menopausal status, race, BMI, and ER status on multiplicative scale

[4] Combined effect of T2D and 4 different effect measure modifiers on additive scale

2.8 FIGURES

Figure. 1a Breast Cancer Specific Survival (BCSS) according to the status of Type 2 diabetes mellitus (T2D): T2D vs. non-T2D

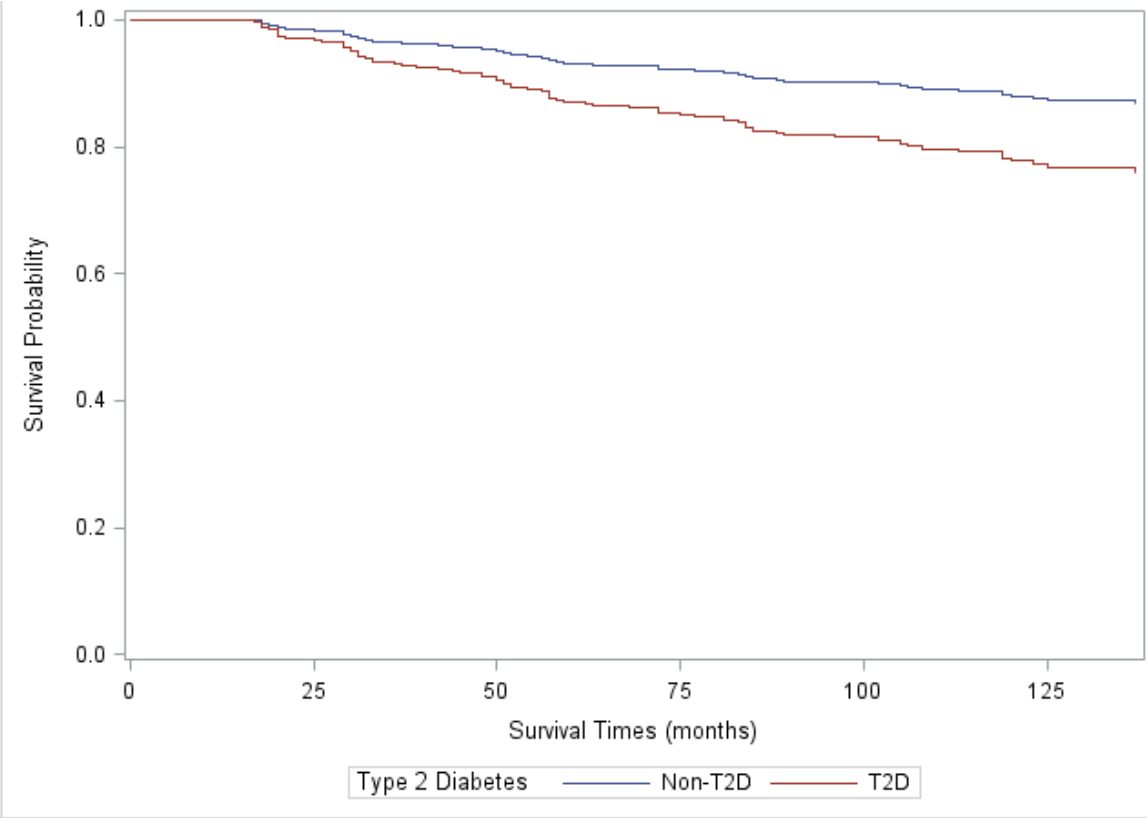


Figure. 1b Overall Survival (OS) according to the status of Type 2 diabetes mellitus (T2D): T2D vs. non-T2D

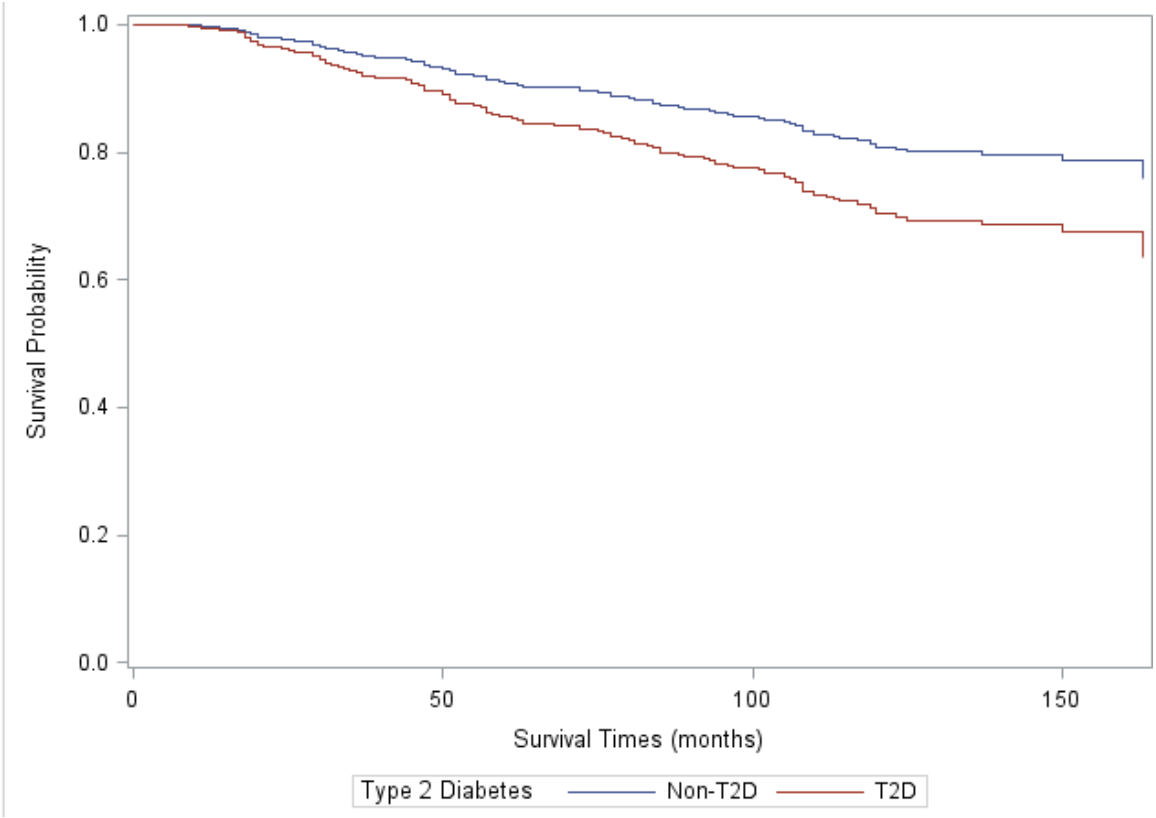


Figure. 2a BCSS according to the type 2 diabetic medication use (metformin vs. no metformin vs. non-T2D)

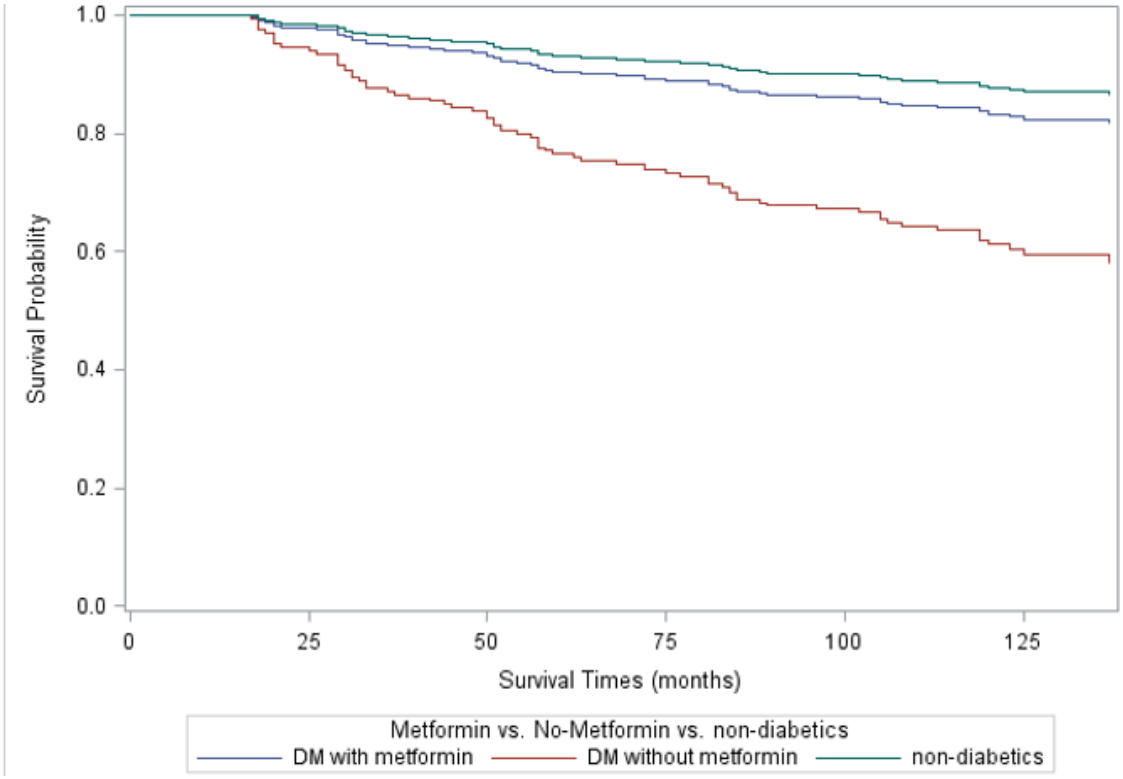
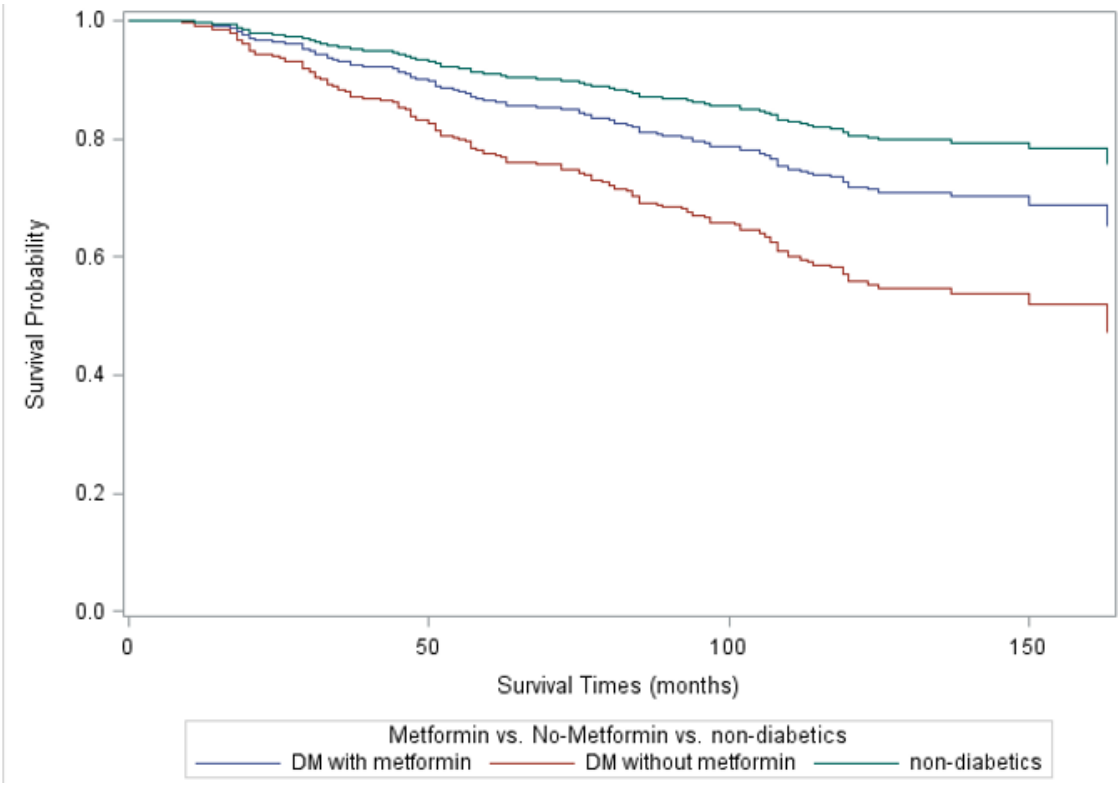


Figure. 2b OS according to the type 2 diabetic medication use (metformin vs. no metformin vs. non-T2D)



CHAPTER III: SUMMARY, PUBLIC HEALTH IMPLICATIONS, POSSIBLE FUTURE DIRECTIONS

Our study assessing the effect of T2D on breast cancer survival outcomes showed that T2D may be a strong prognostic factor for breast cancer-related deaths. While we did not observe interaction with race, BMI, ER, and menopausal status, consistent positive interaction on additive scale (ICR) could suggest the importance of T2D intervention among non-Hispanic blacks, obese, ER negative breast cancer, and pre-menopausal groups to minimize breast cancer-related mortality outcomes. Interaction on the additive scale (rather than multiplicative scale) may be more relevant for biological interaction and directly addresses public health questions of targeted intervention. Our result of lower breast cancer mortality rates among the metformin group compared to the non-metformin group indicated metformin's promising effect as an anti-cancer agent, although larger clinical trials are required.

As the obesity rates increase in the United States, the prevalence of T2D goes up perhaps contributing to the excess risk of deaths among the patients diagnosed with breast cancer. Proper T2D management among the breast cancer patients may be important for prognosis and likely varies across relevant characteristics. Given the racial disparities existing in breast cancer-related deaths, understanding heterogeneous association among breast cancer patients diagnosed with T2D by race, and other important prognostic factors (i.e., BMI, ER, and menopausal status) may be crucial to improve outcomes. Future investigations need to focus on accessing these associations at larger scale to estimate the joint effects of T2D and potential modifiers. These collective data will guide clinicians to develop T2D intervention plans that are specific to high risk groups.

Identifying factors that can increase breast cancer risk and mortality are crucial for public health as it can be used to guide health professionals to provide early screening tools for T2D control among the patients who are newly diagnosed with breast cancer. Integrating T2D control as part of the early management of breast cancer care can help prevent breast cancer-related

morbidity and mortality as well. In addition, clinicians can plan therapeutic treatment guidelines for treating T2D during the early stage of breast cancer in conjunction with breast cancer treatments. Our study indicates potential positive effect of metformin, the first line therapy to treat T2D, to improve BCS survival. T2D intervention using pharmacologic approach may play an important role not only in alleviating T2D related complications but also in preventing breast cancer incidence and mortality that can be aggravated by uncontrolled T2D care. While metformin's anti-cancer benefit has its promises in improving breast cancer survival rates among the T2D patients, metformin's effect does not appear to be the same throughout different population groups as shown from previous breast cancer prevention clinical trials (78, 79). To further determine the causal association between metformin and breast cancer mortality, clinical studies investigating the therapeutic effect of metformin use, dosage, and timing should be conducted in diverse study populations.

In conclusion, our findings have several important public health implications. First, it emphasizes the importance of including proper T2D management in the early stage of breast cancer as there are strong correlation of increased risk of breast cancer deaths among the patients with T2D. Proper education on blood glucose monitoring and intake of diabetic medications need to be reinforced by medical providers in women newly diagnosed with breast cancer. Second, target interventions based on race, body size, ER, or menopausal status may improve outcomes in vulnerable populations. Appropriate guidelines and procedures for T2D management and counseling can be implemented concurrently with breast cancer treatments to help all women newly diagnosed with breast cancer.

4 APPENDICES

4.1 Appendix A

Appendix A. Summary of previous studies examining the effect of T2D on BCS mortality and overall mortality.

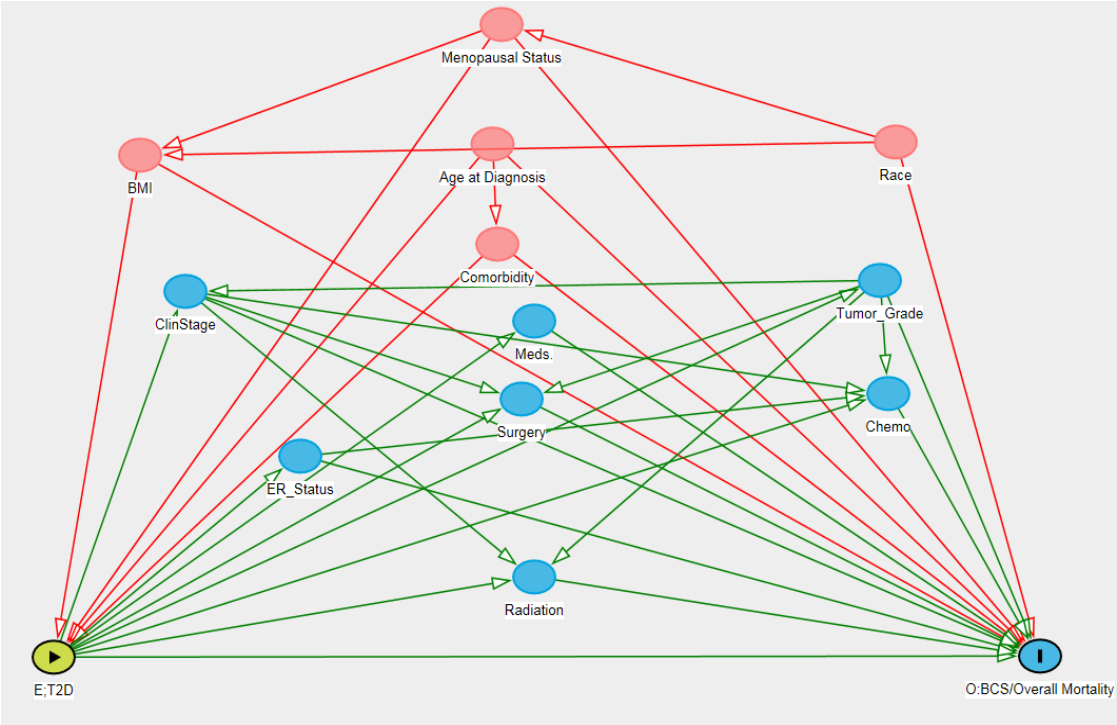
Study Name	**Study Type**	**Population**	**Outcomes** HR/ OR (95% CI)
Luo et al., 2014	Prospective Cohort	- Women's Health Initiative Study - T2D: 727 patients - no-T2D: 7,381 patients	- BCS mortality: HR=0.90 (0.65, 1.24) - Overall mortality: HR=1.26 (1.06, 1.48)
Nechuta et al., 2013	Prospective Cohort	- Shanghai Breast Cancer Survival Study - n= 4,664	- BCS mortality: HR=0.98 (0.68, 1.41) - Overall mortality: HR=1.40 (1.06, 1.85)
De Brujin et al., 2013	Systematic review and meta-analysis consisted of controlled trials, prospective cohort and pooled cohort studies	- 20 studies published after 2007 from Embase, PubMed, and Cochrane library	- BCS mortality: HR=1.38 (1.20, 1.28)
Cleveland et al., 2012	- Population-based study consisted of both the case-control and the follow-up studies	- The Long Island Breast Cancer Study Project (LIBCSP) - 1,508 breast cancer cases & 1,556 control - n=1,508 from follow-up study	- BCS mortality: HR=1.17 (0.63, 2.19) - Overall mortality: HR=1.65 (1.18, 2.29)
Liu et al., 2012	- Population-based Swedish study	- Nationwide Swedish Hospital Discharge Study - T2D: 16,123 patients - no-T2D: 999,982 patients - n= 1,016,105	- BCS mortality: HR=1.45 (1.32, 1.59)

Appendix A. Continued…

Study Name	**Study Type**	**Population**	**Outcomes** HR/ OR (95% CI)
Yeh et al., 2012	- Prospective Cohort Study	- Give Us a Clue to Cancer and Heart Disease (CLUE II) Study - T2D: 116 patients - no-T2D: 2,365 patients - n= 2,481	- BCS mortality: HR=1.27 (0.17, 9.73) - Overall mortality: HR=1.61 (1.29, 2.01)
Srokowski et al., 2009	- Population-based Cohort	- SEER-Medicare linked database from the US National Cancer Institute (NCI) - T2D: 14,414 patients - no-T2D: 56,367 patients	- BCS mortality among patients receiving chemo: OR=1.20 (1.07, 1.35)
Charlot et al., 2017	- Prospective Cohort Study	- Black Women's Health Study - T2D: 232 patients - no-T2D: 1,389 patients	- BCS mortality: HR=1.28 (0.88, 1.86) - Overall mortality: HR=1.54 (1.15, 2.07)
He et al., 2011	- Retrospective Cohort	- University of Texas MD Anderson Cancer Center Database - T2D: 154 patients - no-T2D: 1,829 patients	- Overall mortality: HR=1.42 (1.04, 1.94)
Currie et al., 2012	- Retrospective Cohort	- Anonymous, routine data collected from > 350primary care practices in the U.K. - T2D: 8,392 patients - no-T2D: 104,016 patients - n= 112,408	- BCS mortality: HR=1.32 (1.17-1.49) - Overall mortality: HR= 1.10 (1.07, 1.14)

4.2 Appendix B

Directed acyclic graph (DAG) of the association between T2D (exposure) and BCS mortality and overall mortality (outcome) (80).



4.3 Appendix C

Appendix C. Sensitivity analysis on the effect of T2D on mortality outcomes stratified by BMI only measured at the time of breast cancer diagnosis

	Breast Cancer Specific (BCS) Mortality			Overall Mortality		
		Stratified HR[3]			Stratified HR[3]	
	event/P-month[2]	HR (95% CI)	LRT P-value	event/P-month[2]	HR (95% CI)	LRT P-value
BMI (kg/m2)[1]			0.63			0.79
< 30 (non-obese)						
non-T2D	27/29,421	1.00		44/29,421	1.00	
T2M	3/2,604	1.20 (0.26 to 5.49)		9/2,604	1.18 (0.47 to 2.95)	
≥ 30 (obese)						
non-T2D	13/10,471	1.00		22/10,471	1.00	
T2M	5/2,050	1.86 (0.64 to 5.42)		8/2,050	1.38 (0.61 to 3.15)	

[1] Body Mass Index
[2] events/P-month: number of death occurred by the sum of total time in month contributed by all patients
[3] The effect of T2DM on survival stratified by BMI restricted to the time of breast cancer diagnosis only

4.4 Appendix D

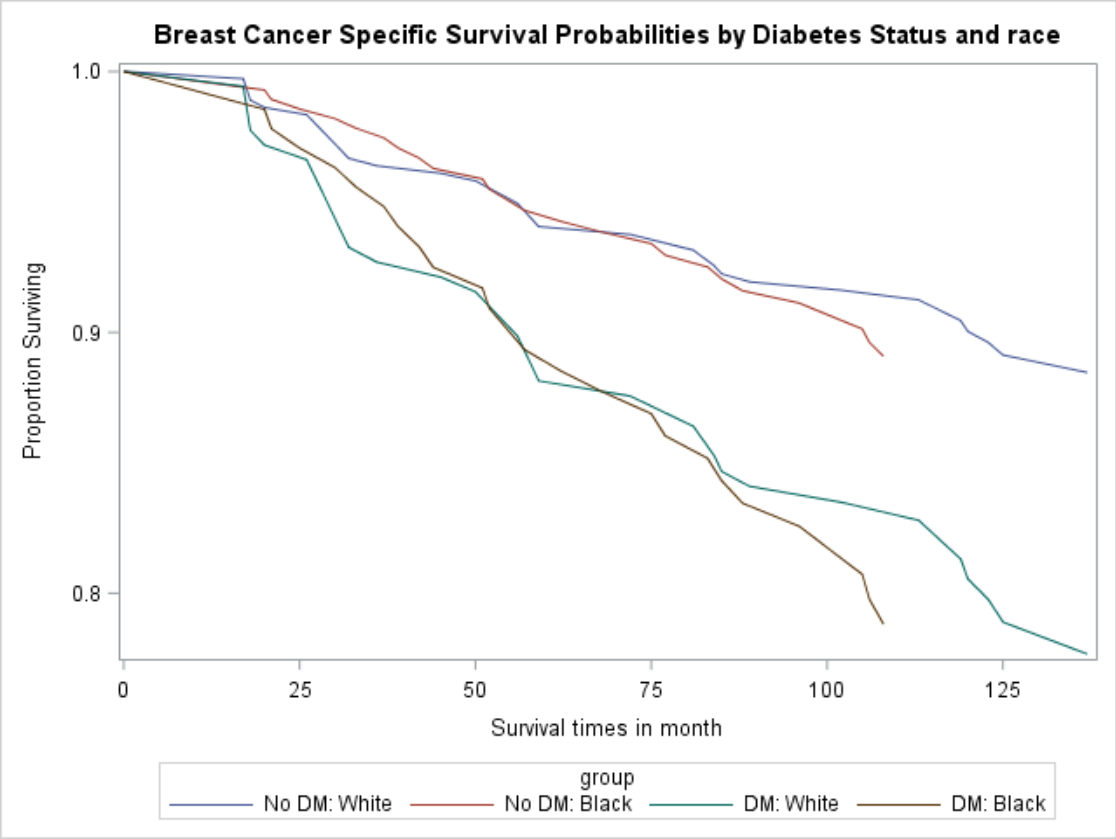
Appendix D. Competing risk analysis using the Fine and Greys Model

	Breast Cancer Specific (BCS) Mortality	
	HR[a]	95% CI
Main effect		
non-T2D	1.00	ref
T2D	1.86	0.99 to 3.47

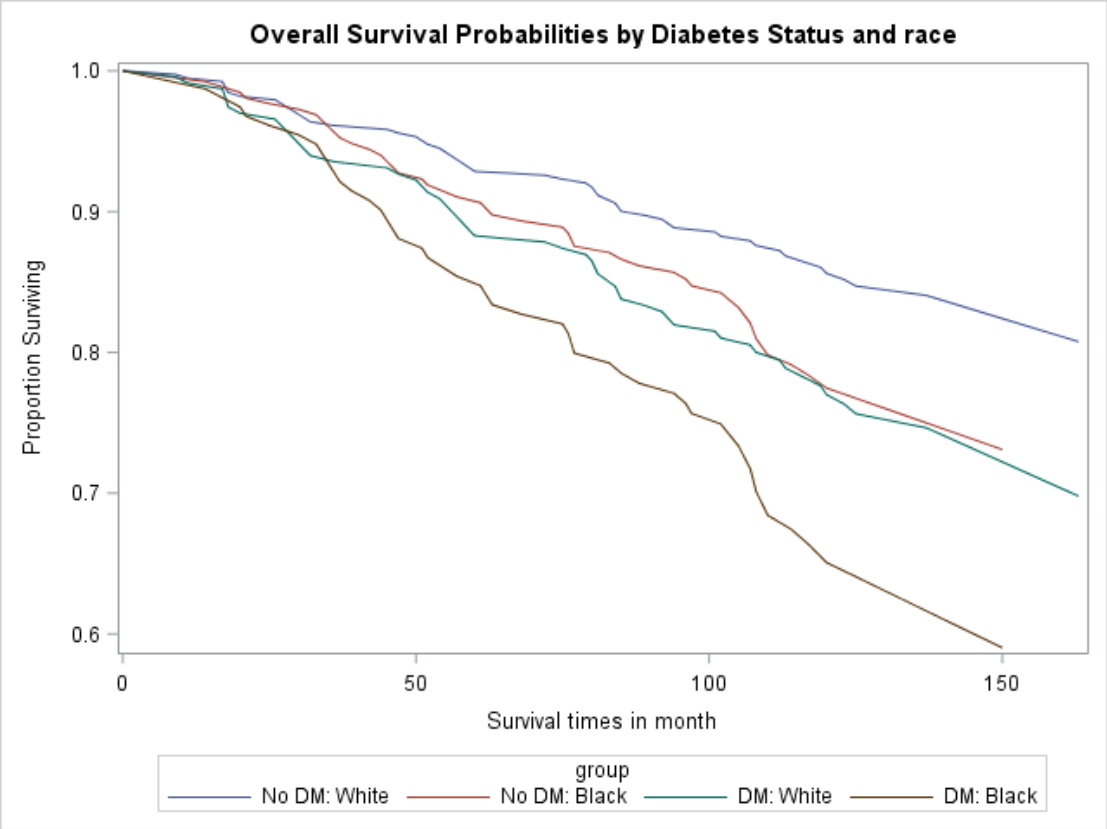
[a] Calculated HR by accounting for competing events of deaths separately

4.5 Appendix E

Appendix E.a. BCSS across different groups by T2D status and race

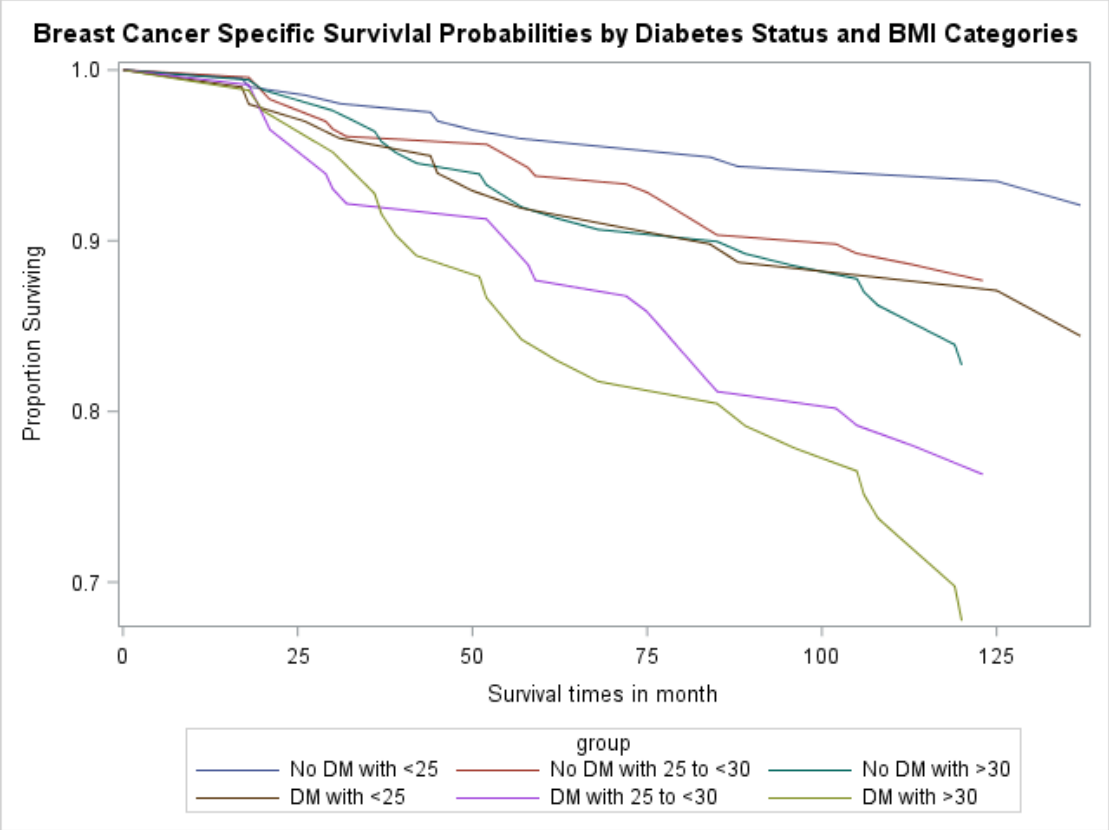


Appendix E.b. OS across different groups by T2D status and race

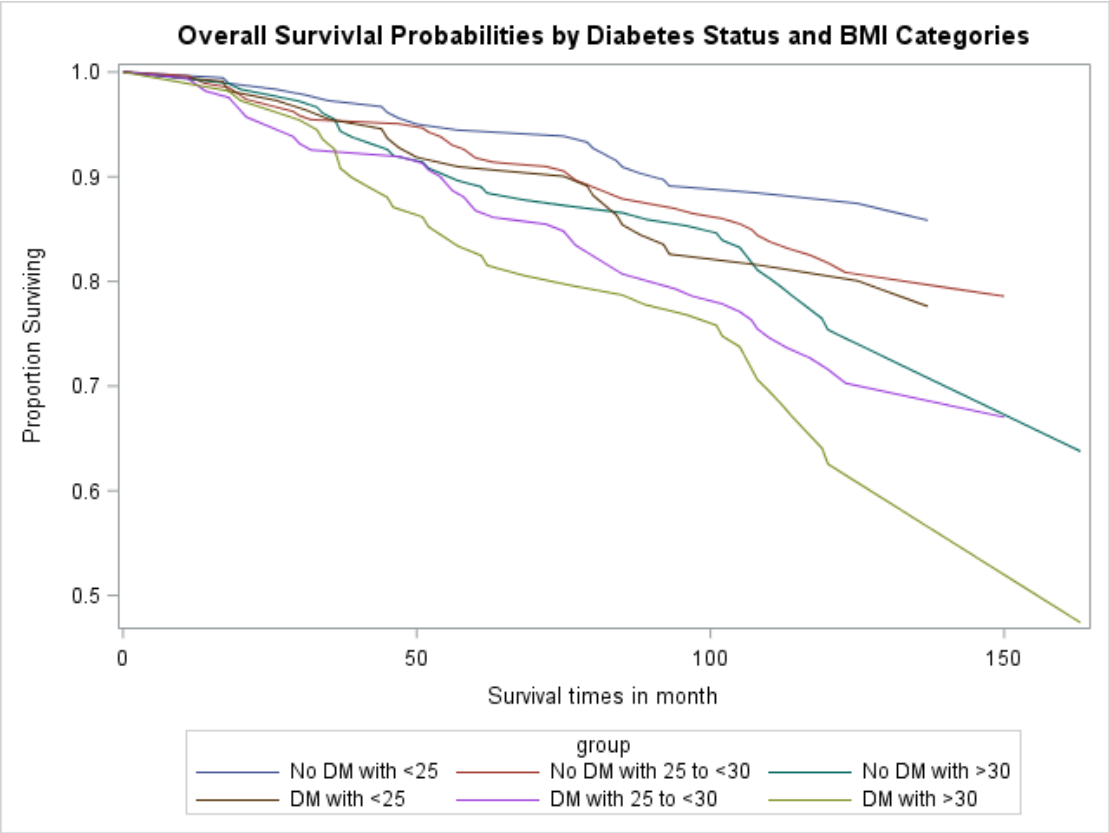


4.4 Appendix F

Appendix F.a. BCSS across different groups by T2D status and different BMI categories

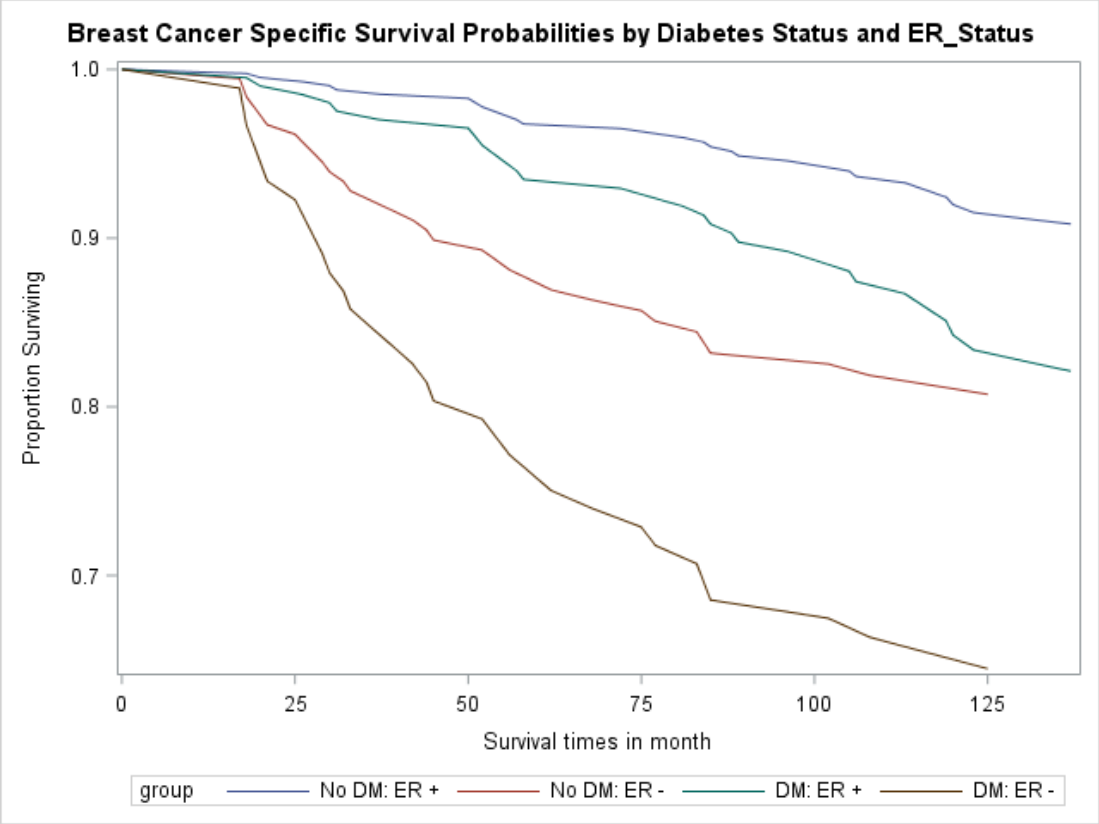


Appendix F.b. OS across different groups by T2D status and different BMI categories

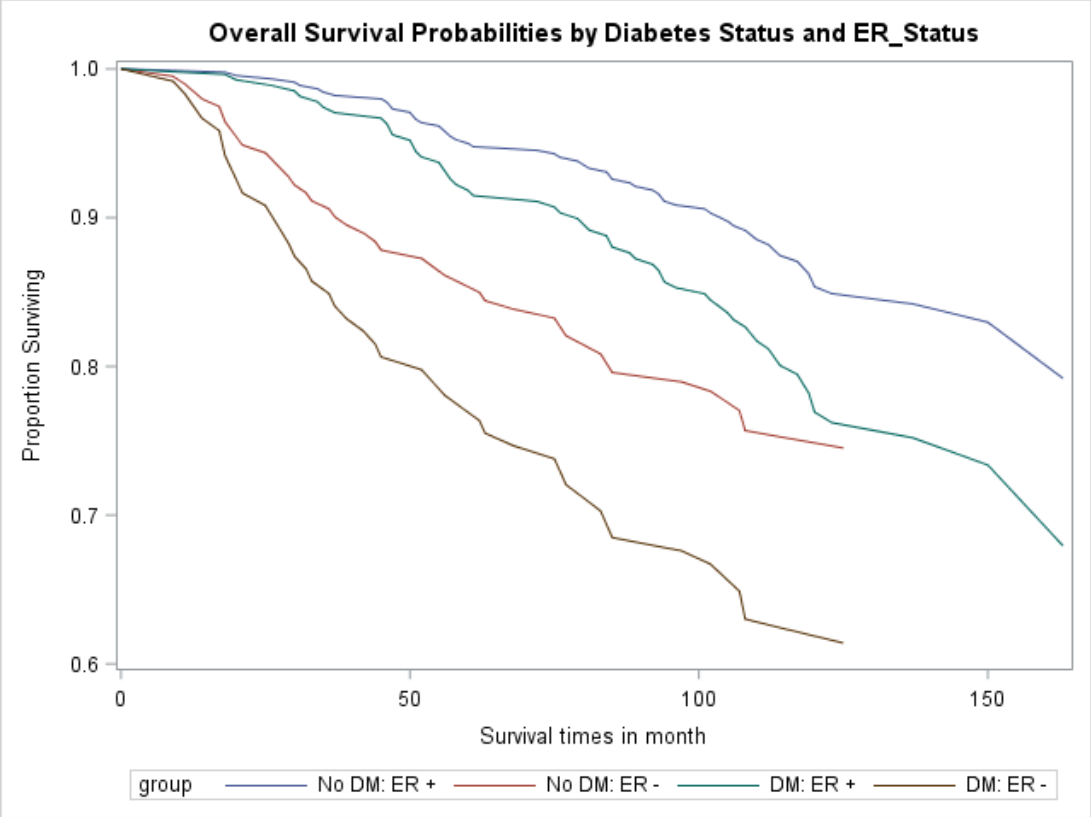


4.5 Appendix G

Appendix G.a. BCSS across different groups by T2D status and ER status

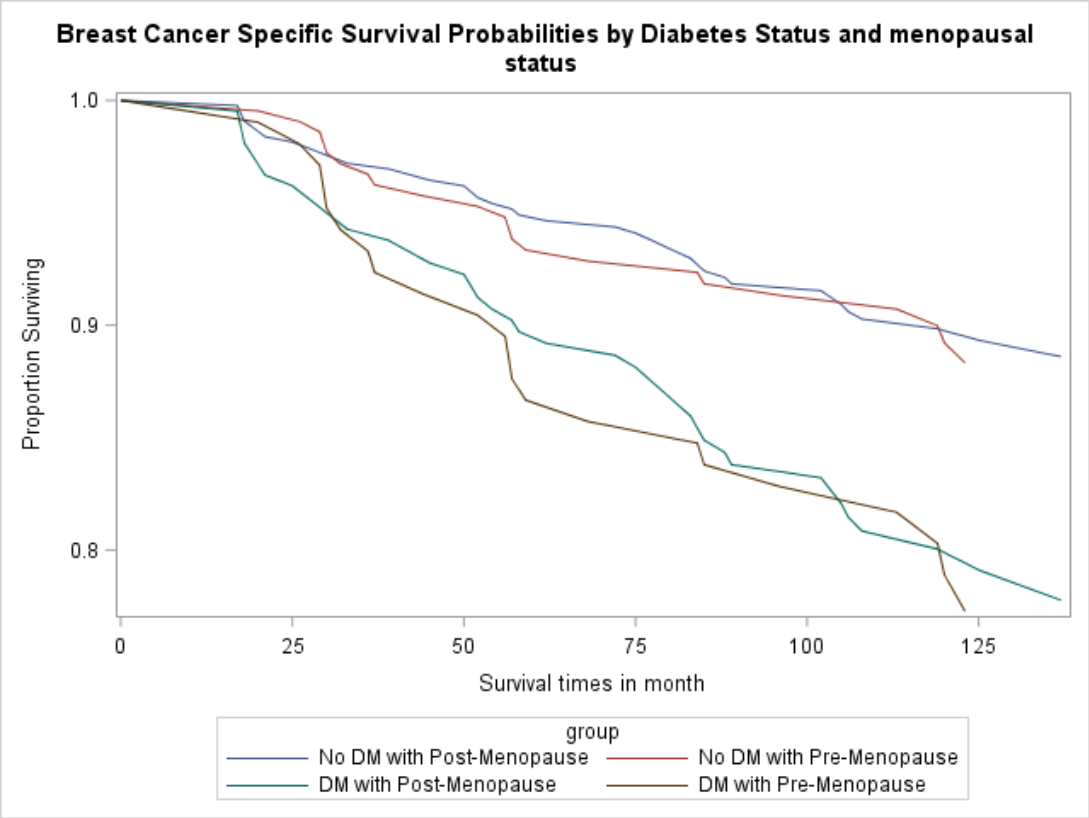


Appendix G.b. OS across different groups by T2D status and ER status



4.6 Appendix H

Appendix H.a. BCSS across different groups by T2D status and menopausal status



Appendix H.b. OS across different groups by T2D status and menopausal status