

Use of Metformin and Survival of Diabetic Women with Breast Cancer

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Abstract: *Objective:* This study was set out to determine whether metformin use influences survival in breast cancer patients treated with antidiabetic drugs as compared to non-users.

Research Design and Methods: We used data from the Danish national registries (1996-2008) to identify adult female patients diagnosed with breast cancer who were prescribed antidiabetic medication. We performed multivariate Cox-proportional hazard regression to assess all-cause and breast cancer-specific mortality risks associated with metformin exposure. In a secondary analysis, we stratified use of metformin according to the cumulative number of prescriptions.

Results: Of the 1058 breast cancer patients 349 died during follow-up, with breast cancer listed as the primary cause of death for 152 cases. Compared to non-use, current metformin treatment was associated with a significant reduction in overall mortality (adjusted HR 0.74, 95% CI, 0.58-0.96). For breast cancer-specific mortality, a non-significant risk reduction (adjusted HR 0.88, 95% CI, 0.59-1.29) was observed, which became significant after stratification according to cumulative number of prescriptions. An increased risk of both overall and breast cancer-specific mortality was observed in the first 12 months after discontinuation of metformin.

Conclusions: We observed a nonsignificant reduction in breast cancer-specific mortality associated with metformin exposure among breast cancer patients treated with antidiabetic drugs. However, our findings suggest that long-term metformin use may have a beneficial effect on survival in patients with breast cancer. Further confirmation of these findings is needed.

Keywords: Breast cancer, metformin, mortality, survival, type 2 diabetes mellitus.

INTRODUCTION

Breast cancer patients with type 2 diabetes mellitus have a higher mortality risk compared with their nondiabetic counterparts [1-4]. Much discussion has recently focused on variations in mortality risk with specific types of antidiabetes medication. Metformin use has been associated with an improved cancer prognosis in observational studies [2, 5, 6] and results from preclinical studies suggest that metformin reduces the growth of breast cancer cells [7-9]. As a relatively safe and inexpensive drug, metformin currently receives much attention as a potential adjuvant to standard breast cancer treatment.

Metformin could decrease breast cancer cell growth, either indirectly by reducing circulating insulin – with known mitogenic effects on breast cancer cell progression *in vitro* [10] – or directly *via* activation of adenosine mono-

phosphate kinase [7-9, 11]. Evidence of reduced tumor cell proliferation from clinical trials, however, is inconclusive [12-14]. Moreover, due to the small numbers of patients, short follow-up periods, and use of surrogate endpoints, findings from clinical trials cannot easily be translated to daily practice.

In addition, results from observational studies on breast cancer survival in diabetic patients are conflicting. Although some fairly small studies reported a beneficial effect of metformin use on survival [15, 16], a recent large and well-designed study failed to show a significant reduction in breast cancer mortality in patients treated with metformin [17]. However, the latter did report on a potential effect associated with cumulative duration of exposure, with a possible 9% reduction (HR 0.91, 95% CI, 0.81-1.03) in breast-cancer specific mortality per additional year of cumulative use [17]. In agreement with these findings, we hypothesized that duration of exposure should be taken into account when evaluating the potential inhibitory effect of metformin use.

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The objective of this study was to determine the relationship between metformin use and all-cause and breast-cancer specific mortality in a cohort of patients diagnosed with breast cancer and treated with antidiabetic drugs and to assess whether this association is dependent upon cumulative duration of exposure.

RESEARCH DESIGN AND METHODS

Data Source and Population

Data for this study were obtained from nationwide registers in Denmark, which include hospital admission records, drug prescriptions, and causes of death for all inhabitants. Linkage of these computerized data is enabled through the use of a personal identification number. Data regarding migration and dates of birth and death are kept by The Ministry of the Interior. All hospital admission records from 1977 onwards are accessible through The National Hospital Discharge Register, which also holds all outpatient visits to hospitals, clinics, and emergency rooms since 1995. The validity of registrations in the database is high and captures almost 100% of contacts [18]. The National Pharmacological Database includes records of all prescription drugs dispensed by pharmacies from 1996 onwards [19], including type of the medication (by ATC code [20]) and dispensing dates [21].

For this study, a cohort was defined from all female patients (aged 18+) receiving treatment for diabetes mellitus who had a first ever diagnostic code for breast cancer (ICD-10 code C50) between 1997 and 2007 [22]. Patients were required to have received at least two prescriptions for a non-insulin antidiabetic drug (NIAD) between 1996 and 2007, of which at least one was dispensed within the year prior to the breast cancer diagnosis. Patients were followed from the moment of breast cancer diagnosis onwards. All patients were required to have a minimum of one year of prescription data available prior to the start of follow-up. Patients with a diagnosis of cancer (except nonmelanoma skin cancer) before the start of follow-up were excluded (n=191). Given the potential mitogenic effect of insulin [23], patients were censored at the time of their first ever insulin (ATC code A10A) prescription. Similarly, patients receiving insulin treatment before the start of follow-up were excluded (n=520). In a sensitivity analysis – adjusted for insulin use in a time-dependent manner, with insulin use defined as prescription of insulin within the past 6 month – we tested the effect of censoring at the time of insulin treatment initiation on the outcome measure. In addition, patients receiving biguanide agents other than metformin (i.e. phenformin, buformin) were excluded (n=2).

Drug Exposure

Exposure to metformin was assessed both before and after breast cancer diagnosis. At start of follow-up (breast cancer diagnosis), baseline metformin exposure was assessed as the number of prescriptions (ATC-code A10BA) in the year preceding the diagnosis. During follow-up, the cumulative number of metformin prescriptions was updated and assessed as a time-dependent variable.

For the time-dependent exposure measurement, time since breast cancer diagnosis was divided into 5-day intervals for each patient and exposure status was updated at start of each (5-day) interval. Current exposure to metformin was defined as a prescription within 3 months prior to the start of an interval. Recent, past, and distant users received their last dispensing in respectively the 3 to 6 months, 6 to 12 months or >1 year before the start of an interval. These categories were mutually exclusive. A patient without a prescription for metformin ever before the start of an interval was considered a “never user” until the time a metformin prescription was filled. To assess cumulative exposure, current use of metformin was stratified according to the total number of prescriptions since 1 year before the index date up to that point. Due to left-truncated data (prescription data was available from 1996 onwards), a proportion of the “distant users” may have been misclassified as “never users”. A sensitivity analysis was performed in which distant use of metformin was relabeled as never use.

Follow-Up and Outcome

Participants were followed from the date of breast cancer diagnosis till the end of data collection (December 31, 2007), emigration, the use of an insulin prescription, or the patient’s death, whichever came first. Data on causes of death were deducted from the death certificate register. If breast cancer was listed as the primary cause, the outcome was labeled as a breast cancer-related death. All other deaths were labeled as breast cancer-unrelated. A sensitivity analysis was performed with breast cancer-unrelated deaths as the study outcome.

Other Covariates

Information regarding *a priori* risk factors for breast cancer prognosis was collected and incorporated in the analysis. The presence of risk factors was updated at the start of each 5-day interval and analyzed as time-dependent covariates. Age was included as a continuous variable. Other potential confounders included the Charlson Comorbidity Index (based on a history of chronic diseases, including amongst others cerebrovascular disease, congestive heart failure, and ischemic heart disease [24]), and the use of concomitant medication: i.e. sulfonamides (A10BB and A10BC), thiazolidinediones (A10BG), other glucose-lowering agents (A10BF, alfa-glucoside inhibitors; A10BH, dipeptidyl peptidase 4 inhibitors), statins (C10AA), and hormone replacement therapy (G03FB). Exposure to comedication was defined as a prescription within the past 6 months. To adjust for variations in breast cancer treatment over time, the number of years between the start of the study period (January 1, 1997) and the date of breast cancer diagnosis was included as a continuous variable.

Statistical Analysis

Patient characteristics and risk factors for breast cancer prognosis available in the dataset were compared between patients treated with metformin at the time of breast cancer diagnosis and those receiving other non-insulin antidiabetic treatment. Cox-proportional hazards models were estimated to evaluate the effect of current, past, and distant past exposure to metformin versus never use on all-cause and

breast cancer-specific mortality. In a secondary analysis, current use of metformin was differentiated according to the cumulative number of prescriptions since 1 year before the index date. The association between metformin exposure and the study outcome was adjusted for all specified potential confounders in a time-dependent manner. All statistical analyses were performed with SAS (version 9.2).

RESULTS

Study Population

The study cohort consisted of 1058 subjects who were diagnosed with breast cancer and were dispensed at least one prescription for an antidiabetic drug in the year prior to the diagnosis, with a total follow-up of 2971 person-years. Table 1 shows the baseline characteristics of patients treated with metformin within the year prior to the breast cancer diagnoses compared to those who were not treated with metformin. Subjects treated with metformin were younger and more likely to receive concomitant statin treatment. Patients not receiving metformin were primarily treated with sulfonylurea monotherapy. Cumulative number of metformin prescriptions within 365 days before diagnosis varied between 1 and 27. Overall co-morbidity was comparable; besides complications resulting from type 2 diabetes, the most common co-morbidities in both groups were cerebrovascular disease, congestive heart failure, and ischemic heart disease. Insulin treatment was started earlier in patients not treated with metformin, resulting in a 51% reduction in follow-up time after censoring. For patients treated with metformin, follow-up time was reduced by 31%.

In total, 349 patients (33.0%) died within the study period. Of those, cancer was listed as the primary cause of death for 172 cases (49.3%) and for the vast majority of cancer deaths, patients died of breast cancer (n=152, 88.4%). After breast cancer, the most common cause of death was cardiovascular disease (n=74, 21.2%). Forty-four (12.6%) death certificate records did not specify cause of death.

Effect of Metformin Use on All-Cause Mortality

As shown in Table 2, current use of metformin was associated with a significant reduction in all-cause mortality (adjusted HR 0.74, 95% CI, 0.58-0.96). Differentiation according to the number of prescription revealed that the reduction was most profound for the categories with the highest cumulative exposure. However, the differences between prescription categories failed to reach statistical significance for any direct comparisons. Conversely, recent and past use were both associated with a significant increased overall mortality risk, while no difference was observed between never use and distant use of metformin.

Effect of Metformin Use on Breast Cancer-Specific Mortality

Table 3 shows a non-significant risk reduction in breast cancer-specific mortality was observed in association with current use of metformin as compared to nonuse (adjusted HR 0.88, 95% CI, 0.59-1.29). Differentiation according to the number of prescriptions showed a noticeable fluctuation in risk: while the lowest two categories were associated with

a non-significant increased breast cancer mortality (adjusted HR 1.39, 95% CI, 0.73-2.65 and adjusted HR 1.29, 95%

Table 1. Baseline Characteristics of Diabetic Women with Breast Cancer, by Metformin Exposure in the Year Prior to Diagnosis

Characteristic	Metformin (n=508)		Nonmetformin (n=550)	
Follow-up (yrs)	1280.5		1690.2	
Median (IQR)	1.8	(0.8-3.8)	2.6	(0.9-4.4)
Age at BC diagnosis				
Median (IQR)	68	(60-76)	76	(67-82)*
By category (%)				
<50	23	(4.5)	9	(1.6)†
50-60	94	(18.5)	41	(7.5)†
60-70	165	(32.5)	122	(22.2)†
70-80	152	(29.9)	189	(34.4)
80-90	70	(13.8)	160	(29.1)†
90+	4	(0.8)	29	(5.3)†
Year of BC diagnosis				
<2000	75	(14.8)	179	(32.5)†
2000-2005	279	(54.9)	293	(53.3)
>2005	154	(30.3)	78	(14.2)†
Charlson Comorbidity Index				
Median (IQR)	3	(3-4)	3	(3-5)‡
By category (%)				
3	307	(60.4)	294	(53.5)†
4-5	139	(27.4)	184	(33.5)†
6-8	46	(9.1)	57	(10.4)
≥9	16	(3.2)	15	(2.7)
Medication in previous year				
Metformin	508	(100.0)	-	-
By number of Rx				
Median (IQR)	6	(4-8)	-	-
1-5	219	(43.1)	-	-
6-10	216	(42.5)	-	-
> 10	73	(14.4)	-	-
SU-derivates	288	(56.7)	541	(98.4)†
Thiazolidinediones	11	(2.2)	6	(1.1)
Other NIAD‡	17	(3.4)	23	(4.2)
HRT	85	(16.7)	106	(19.3)
Statins	197	(38.8)	115	(20.9)†

IQR, interquartile range; NIAD, non-insulin antidiabetic drug, HRT, hormone replacement therapy. *Statistically significant difference ($p<0.05$), based on Mann-Whitney-Wilcoxon test. †Statistically significant difference ($p<0.05$) between metformin and nonmetformin groups, based on Chi-square test. ‡Glucagon-like peptide-1 agonists, alpha glucosidase inhibitors, meglitinides, and dipeptidyl peptidase 4-inhibitors.

Table 2. All-Cause Mortality Associated with Use of Metformin in Female Diabetic Breast Cancer Patients

	Incidence Rate			Hazard Ratios			
	Events	py	Events/1000 py	Age Adj.	95% CI	Fully Adj. [*]	95% CI
Never [†]	176	1150.1	153.0	1	ref.	1	ref.
Current [‡]	112	1457.0	76.9	0.72	[0.56-0.93]	0.74	[0.58-0.96]
Number of Rx [§]							
1 to 5	19	169.3	112.2	0.99	[0.62-1.60]	1.09	[0.67-1.76]
6 to 10	26	263.1	98.8	0.90	[0.59-1.37]	0.86	[0.56-1.31]
11 to 21	31	420.8	73.7	0.71	[0.48-1.06]	0.68	[0.46-1.02]
21 to 31	14	243.2	57.6	0.56	[0.32-0.97]	0.55	[0.31-0.96]
>30	22	360.5	61.0	0.55	[0.35-0.87]	0.64	[0.40-1.05]
Recent	18	86.1	209.1	1.91	[1.17-3.13]	1.75	[1.07-2.88]**
Past [¶]	14	56.4	248.2	1.96	[1.13-3.39]	1.85	[1.07-3.21]**
Distant [#]	29	221.0	131.2	0.99	[0.67-1.47]	0.98	[0.65-1.46]

HR, hazard ratio; py, person years; adj., adjusted; CI, confidence interval; ref., reference category; Rx, prescriptions. *Adjusted for all potential confounders (age, Charlson Comorbidity Index, number of years between January 1, 1997 and the date of breast cancer diagnosis, and use of concomitant medication during follow-up: metformin, sulfonylureas, thiazolidinediones, other antidiabetic drugs, hormone replacement therapy, and statins in the past 6 months). †No prior recorded prescription for metformin. ‡Metformin prescription in past 3 months. §Total number of prescriptions for metformin since 1 year before breast cancer diagnosis. ||Last metformin prescription within past 3 to 6 months. ¶Last metformin prescription within past 6 to 12 months. #Last metformin prescription more than 1 year ago. **Statistically significant difference ($p < 0.05$) with current use of metformin, based on Wald test.

Table 3. Breast Cancer-Specific Mortality Associated with Use of Metformin in Female Diabetic Breast Cancer Patients

	Incidence Rate			Hazard Ratios			
	Events	py	Events/1000 py	Age Adj.	95% CI	Fully Adj. [*]	95% CI
Never [†]	75	1150.1	65.2	1	ref.	1	ref.
Current [‡]	51	1457.0	35.0	0.83	[0.57-1.22]	0.88	[0.59-1.29]
Number of Rx [§]							
1 to 5	11	169.3	65.0	1.32	[0.69-2.49]	1.39	[0.73-2.65]
6 to 10	18	263.1	68.4	1.44	[0.85-2.45]	1.29	[0.76-2.21]
11 to 21	16	420.8	38.1	0.92	[0.53-1.61]	0.87	[0.49-1.53]
21 to 31	2	243.2	8.2	0.20	[0.05-0.81]	0.20	[0.05-0.84]**
>30	4	360.5	11.1	0.28	[0.10-0.77]	0.38	[0.13-1.09]**
Recent	11	86.1	127.8	2.84	[1.49-5.41]	2.58	[1.35-4.96]††
Past [¶]	7	56.4	124.1	2.42	[1.10-5.30]	2.23	[1.01-4.91]††
Distant [#]	8	221.0	36.2	0.72	[0.34-1.50]	0.79	[0.38-1.67]

HR, hazard ratio; py, person years; adj., adjusted; CI, confidence interval; ref., reference category; Rx, prescriptions. *Adjusted for all potential confounders (age, Charlson Comorbidity Index, number of years between January 1, 1997 and the date of breast cancer diagnosis, and use of concomitant medication during follow-up: sulfonylureas, thiazolidinediones, other antidiabetic drugs, hormone replacement therapy, and statins in the past 6 months). †No prior recorded prescription for metformin. ‡Metformin prescription in past 3 months. §Total number of prescriptions for metformin since 1 year before breast cancer diagnosis. ||Last metformin prescription within past 3 to 6 months. ¶Last metformin prescription within past 6 to 12 months. #Last metformin prescription more than 1 year ago. **Statistically significant difference ($p < 0.05$) with 1 to 5 and 6 to 10 prescriptions for metformin, based on Wald test. ††Statistically significant difference ($p < 0.05$) with current and distant past use of metformin, based on Wald test.

CI, 0.76-2.21, respectively), a decrease in risk was observed for a cumulative number of prescriptions between 21 and 30 and for the highest category (adjusted HR 0.20, 95% CI, 0.05-0.84 and HR 0.38, 95% CI, 0.13-1.09, respectively). Moreover, significant differences in breast cancer mortality risk were observed between the lower and higher exposure categories. The increased risk associated with recent and past use was more pronounced for breast cancer mortality than observed in the analysis on all-cause mortality. When the last

metformin prescription was filled over 1 year ago, the risk was similar to patients never exposed to metformin.

Sensitivity Analyses

A sensitivity analysis with breast cancer-unrelated deaths ($n=197$) as the study outcome revealed that current metformin use was associated with a reduced mortality risk (adjusted HR 0.66, 95% CI, 0.47-0.93). A non-significant

increased risk was observed for recent and past use of metformin (adjusted HR 1.16, 95% CI, 0.53-2.54 and adjusted HR 1.59, 95% CI, 0.73-3.45). Stratification according to the cumulative number of prescription showed a protective effect associated with all prescription categories for mortality by causes other than breast cancer, with no significant differences between categories. In addition, relabeling of distant use as never use of metformin did not affect the results as they are presented in Tables 2 and 3 in any significant way. Likewise, the results of the sensitivity analysis regarding the effect of censoring at the time of insulin treatment initiation showed similar results (Data not shown).

DISCUSSION

In this study we found that current metformin use was associated with a significant reduction in overall mortality (adjusted HR 0.74, 95% CI, 0.58-0.96), but not in breast cancer-specific mortality (adjusted HR 0.88, 95% CI, 0.59-1.29). After stratification according to the cumulative number of prescriptions, the categories with the highest cumulative metformin use appeared to be associated with lower breast cancer mortality. Unexpectedly, a significant increase in both overall and breast cancer-specific mortality was observed between 3 and 12 months after the last metformin prescription.

The present findings are consistent with the results of a recent population-based study by Lega *et al.*, (2013). Although their findings did not reach statistical significance, Lega *et al.*, reported a possible 9% reduction in breast cancer-specific mortality per additional year of cumulative metformin use [17], suggesting the beneficial effect of metformin use may be dependent on duration of treatment. In agreement with this hypothesis, we observed an inverse relationship between the cumulative number of prescriptions and breast cancer-specific mortality, where the highest cumulative use appeared to be associated with a reduced risk. Moreover, our results coincide with regard to the specificity of this duration response effect; like Lega *et al.*, the effect of cumulative duration of use in our study was most pronounced for breast-cancer specific mortality.

Comparison with results from other observational studies concerning the effect of metformin use on survival in breast cancer patients is hindered by several limitations. In two relatively small observational studies, concerning specific breast cancer subtypes, metformin use associated with improved cancer-specific [16] and with a lower risk of distant metastases [25]. However, exposure to antidiabetic treatment in these two studies was defined by use at the time of diagnosis or any use during follow-up, potentially introducing immortal time bias [26].

Results from preclinical studies suggest that metformin may decrease breast cancer cell growth, either by reducing circulating insulin or through direct activation of AMPK [7-9, 11]. Small randomized controlled clinical trials indicate that metformin treatment causes significant reductions in surrogate endpoints (Ki-67 levels) [13, 14]. Furthermore, the effect appeared to be modified by insulin resistance status [12-14], indicating metformin may affect breast cancer prognosis mainly by lowering circulating insulin levels [12,

14]. Insulin has known mitogenic effects on mammary tissue and breast cancer cells *in vitro* [10].

Nevertheless, our findings are not fully in line with the suggested mechanism of action. Patients receiving treatment with metformin in the year preceding the breast cancer diagnosis were significantly younger at the time of diagnosis. If metformin slows cell growth, age at diagnosis would expectedly be higher in patients treated with metformin. However, the inverse relationship between breast cancer mortality and the cumulative number of prescriptions suggests that the improved survival associated with metformin use is not simply caused by selection bias. This notion is supported by the observation that the relative risk of all-cause and breast cancer-unrelated mortality did not show significant differences between prescription categories. Based on these findings, we cannot rule out nor confirm that the significant protective effect for all-cause mortality is the result of selection bias, while the reduction in breast cancer-specific mortality for the upper categories of cumulative use is a true effect of metformin exposure. However, the observed duration response relationship for breast cancer-specific mortality provides valid evidence that our findings did not result from a fundamental flaw in study design. Moreover, as we used Cox proportional hazard analyses, the duration of follow-up is held constant within the comparison between categories of cumulative number of prescriptions.

We can only speculate on an explanation for the increase in breast cancer-specific mortality observed between 3 and 12 months after the last metformin prescription. Drawing on postulated theories regarding metformin's actions, no harmful effects of discontinuation were expected. More peculiar, the pattern of increased mortality risk after discontinuation of metformin seemed to be restricted to breast cancer-specific mortality. An explanation for this finding could entail a reduction in medication burden or increased weight loss in late-stage cancer patients, leading to discontinuation of (some) antidiabetic medication. Metformin treatment may also be stopped out of caution to prevent lactic acidosis. In addition, hospital admission (or admission to a palliative care unit) of end-stage cancer patients may lead to non-observable prescription data. Lastly, active cancer may destabilize glucose metabolism, causing switches in antidiabetic treatment that could result in protopathic bias. Unfortunately, we did not have sufficient information to test any of these hypotheses.

Our study is subject to several limitations that should be taken into consideration when interpreting these findings. First of all, we did not perform a competing risk analysis. However, as metformin use was associated with a significant reduction in breast cancer-unrelated mortality, patients currently treated with metformin had a lower risk of being censored due to a competing risk. If anything, this may have biased our results towards an increased breast cancer-specific mortality associated with metformin use. As metformin users were younger at the time of diagnosis, they have a better initial prognosis. However, all analyses were adjusted for age at diagnosis; the consequential bias towards a protective effect of metformin is minimized. Furthermore, the current cohort incorporated mainly postmenopausal women and our findings are only relevant to this particular patient population. Moreover, due to the definition of the inclusion

criteria, the cohort comprised only individuals treated with NIADs who, if not treated with metformin, had to be treated with another NIAD. Consequently, confounding by indication may have influenced our results. Since metformin is a commonly used first-line drug, this may have biased our results towards a protective effect [26]. However, an exaggerated protective effect stemming from this type of bias would not be influenced by cumulative use. In addition, cumulative number of prescriptions is a crude measure of duration of use, since time between prescriptions can vary between individuals. Lastly, we were unable to adjust for several potential confounders (e.g. tumor staging at the time of diagnosis, cancer treatment, body-mass index, smoking, social deprivation, menopause status), since these data were not available. However, a recent study found no difference in tumor stage or nuclear grade between diabetic patients treated with metformin and those treated with other antidiabetic drugs [15]. Based on these findings, we also do not expect cancer treatment to vary by concomitant use of specific types of NIADs.

Strengths of this study comprise the large number of patients included and the use of population-based data. Furthermore, data are of high quality and objectively gathered from the population. By using pharmacy prescription data, we were able to assess metformin use as a time-dependent variable which prevents immortal time bias. In addition, by restricting the study population to patients treated with oral antidiabetic drugs, we attempted to select relatively comparable patients with respect to disease burden. Moreover, data from death certificates allowed for differentiation between breast cancer deaths and deaths by other causes. Further, while overall mortality is expected to be susceptible to confounding by indication (i.e. metformin use may be a proxy for overall better health), cancer specific-mortality may be more robust. Lastly, our study incorporated a measure of duration of exposure (measured by number of prescriptions) to assess any duration response effect.

In summary, our study provides further evidence that duration of use is relevant when evaluating the clinical relevance of metformin in breast cancer treatment. However, unexpected findings with regard to an increased mortality after discontinuation and an apparent increased risk associated with short-term use of metformin necessitate additional confirmatory studies.

AUTHOR CONTRIBUTIONS

Study design was formulated by MLDB, FdV, MTB, and PJHLP. The latter, in collaboration with MLDB and MTB, was responsible for data analysis and writing of the paper. Other authors contributed important comments and insights with regard to the interpretation of results. All authors were involved in safeguarding academic standards and highlighting potential biases.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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PATIENT CONSENT

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