Original Article

Effect of Metformin on Survival Outcomes In Diabetic Patients With Triple Receptor-Negative Breast Cancer

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BACKGROUND: Recent observational studies have shown that metformin use in diabetic patients decreases both cancer incidence and mortality. Metformin use is also independently predictive of pathologic complete response. In the current study, the authors explored the association between metformin use and survival outcomes in patients with triple receptor-negative breast cancer (TNBC) who were receiving adjuvant chemotherapy. **METHODS:** The Breast Cancer Management System database of The University of Texas MD Anderson Cancer Center identified 1448 women who received adjuvant chemotherapy for TNBC between 1995 and 2007. Patients were categorized by diabetes status and metformin use. The Kaplan-Meier product-limit method was used to calculate distant metastasis-free survival (DMFS), recurrence-free survival (RFS), and overall survival (OS). Cox proportional hazards models were fit to determine the association between metformin use and survival outcomes. **RESULTS:** The study cohort was comprised of 63 diabetic patients receiving treatment with metformin, 67 diabetic patients not receiving metformin, and 1318 nondiabetic patients. Patients in the diabetic groups tended to be older ($P = .005$); more diabetic patients were postmenopausal ($P = .0007$), black ($P = .0001$), and obese ($P < .0001$). At a median follow-up of 62 months, there were no significant differences with regard to 5-year DMFS ($P = .23$), RFS ($P = .38$), and OS ($P = .58$) between the 3 groups. Compared with the metformin group, patients who did not receive metformin (hazard ratio [HR], 1.63; 95% confidence interval [95% CI], 0.87-3.06 [$P = .13$]) and nondiabetic patients (HR, 1.62; 95% CI, 0.97-2.71 [$P = .06$]) tended to have a higher risk of distant metastases. **CONCLUSIONS:** The findings of the current study suggest that metformin use during adjuvant chemotherapy does not significantly impact survival outcomes in diabetic patients with TNBC. ***Cancer* 2012;118:1202–11.** © *2011 American Cancer Society.*

KEYWORDS: triple receptor-negative breast cancer, metformin, diabetes mellitus, survival, recurrence, adjuvant chemotherapy.

INTRODUCTION

It is believed that 8% to 18% of cancer patients have diabetes.[1] The increased coexistence of diabetes with breast cancer has been related to the mitogenic effects of signaling through the insulin/insulin-like growth factor 1 (IGF1) and insulin receptor.[2,3] Diabetes mellitus is believed not only to be a moderate risk factor for breast cancer but also to considerably worsen prognosis in patients with this disease.[4,5]

Metformin is a widely prescribed oral hypoglycemic agent that is used as first-line therapy for patients with type 2 diabetes mellitus. Several studies have suggested a possible association between the use of metformin and a decreased risk of cancer,[6-9] and cancer-specific mortality in diabetic patients.[6,10] The mechanisms involved in the antiproliferative effects of metformin are most likely very diverse.[11,12] Preclinical studies with breast cancer cell lines have demonstrated that metformin acts as a growth inhibitor that is mediated by upregulation of AMP-activated protein kinase (AMPK) activity and downstream suppression of signaling through the mammalian target of rapamycin (mTOR).[13] Other mechanisms include, in particular, influences on estrogen biosynthesis and estrogenic signal transduction[14-16] and suppression of

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human epidermal growth factor receptor-2 (HER-2) protein expression.[17] More recently, Liu et al[18] demonstrated unique apoptotic effects of metformin against triple receptor-negative breast cancer (TNBC) cell lines via poly (ADP-ribose) polymerase (PARP) cleavage and the activation of both intrinsic and extrinsic caspase signaling cascades. Moreover, in nude mice, metformin modestly inhibited tumor growth of xenografts of a TNBC cell line.[18]

Recent observational studies have shown that metformin use in patients with diabetes decreases both cancer incidence and mortality.[6,10] Strikingly, this effect appeared to improve with higher doses.[6,19] Despite these promising results, to the best of our knowledge there is only sparse evidence from clinical studies addressing the relative impact of metformin on the outcome of patients with breast cancer. Furthermore, it is not clear whether metformin use is predictive of improved long-term survival outcomes.

The objective of the current study was to investigate in a retrospective setting the distant metastasis-free survival (DMFS), recurrence-free survival (RFS), and overall survival (OS) outcomes among diabetic patients receiving treatment with metformin, diabetic patients not receiving metformin, and nondiabetic patients.

MATERIALS AND METHODS

Patient Population

The Breast Cancer Management System database of The University of Texas MD Anderson Cancer Center (MDACC) identified 1448 women who were diagnosed and treated with adjuvant chemotherapy for TNBC between 1995 and 2007. Patients with metastatic or bilateral disease, a prior history of cancer, resolved gestational diabetes, or diabetes diagnosed after the period of adjuvant chemotherapy were excluded from the analysis.

This study was approved by the Institutional Review Board at MDACC. Accuracy of clinical variables recorded within the prospectively collected data was verified by retrospective review of individual patient records.

Pathologic Evaluation

All pathologic specimens were reviewed by dedicated breast pathologists at MDACC. Invasive carcinoma was confirmed on initial core needle biopsy specimens. The initial clinical stage and pathologic stage of disease in all patients were revised and based on the seventh edition of the American Joint Committee on Cancer staging criteria.[20] Histologic type and grade were defined according to the World Health Organization classification system[21] and modified Black nuclear grading system,[22] respectively. Negative estrogen receptor (ER) and progesterone receptor (PR) status was defined as nuclear staining of $\leq$ 10% on immunohistochemical (IHC) analysis. HER-2–negative status was defined as either 1+ or no staining by IHC and/or the absence of gene amplification by fluorescence in situ hybridization.

Treatment

The type of surgery undertaken and adjuvant chemotherapy received were at the discretion of the patient and multidisciplinary treating team. Surgical intervention was breast-conserving surgery (BCS) for 51% of patients (n = 738) and mastectomy for 49% of patients (n = 710). Adjuvant chemotherapy regimens were comprised of anthracycline-based regimens with a taxane (758 patients; 45%) or without a taxane (675 patients; 40%), single-agent taxane (65 patients; 4%), and non–anthracycline-containing/non–taxane-containing regimens (181 patients; 11%). Postoperative radiotherapy was administered if patients had undergone BCS, had locally advanced disease at the time of presentation, had a primary tumor measuring $>$ 5 cm, or had $\geq$ 4 involved axillary lymph nodes. None of the patients received adjuvant endocrine therapy.

Statistical Analysis and Outcome Measures

The demographic and clinical characteristics of the 3 study groups were compared with the chi-square test. DMFS, RFS, and OS were defined as the time from the date of definitive surgery until the first date of documented distant metastasis, disease recurrence, or death from any cause, respectively. Patients not experiencing the relevant endpoints were censored at the time of last follow-up. Survival outcomes were estimated with the Kaplan-Meier product-limit method; groups were compared using the log-rank statistic. Cox proportional hazards models were fitted to determine the association between the use of metformin and survival outcomes after adjustment for age ($>$ 50 years vs $\leq$ 50 years), tumor T classification (T3-4 vs T1-2), lymph node classification (N1-3 vs N0), nuclear grade (3 vs 1/2, lymphovascular invasion (LVI) (positive vs negative), and chemotherapy type (anthracycline-based chemotherapy vs taxane-based chemotherapy vs anthracyclines and taxane-based chemotherapy vs other). Factors included in the multivariate models for adjustment were based on both statistical and

clinical significance. Hazard rate and ratio values were estimated using kernel functions.[23] Statistical analyses were performed using SAS (version 9.1; SAS Institute Inc, Cary, NC) and S-Plus (version 7.0; Insightful Corporation, Seattle, WA) statistical software.

RESULTS

Patient Demographics and Clinical Characteristics

A total of 1448 patients were included in the statistical analysis, 130 of whom (9%) were diabetic and 1318 of whom (91%) were nondiabetic. Of all the diabetic patients, 63 (48%) were receiving metformin during adjuvant chemotherapy (metformin group). In the nonmetformin group, glycemic control was achieved using different modes of antidiabetic therapy, including dietary treatment only (30%), sulfonylurea preparations (28%), insulin therapy (39%), or thiazolidinediones (20%). Patient demographics and clinical characteristics including comorbidities and concomitant medications are summarized in Table 1. Patients in the diabetic groups were older ($P = .005$) than those in the nondiabetic group. The diabetic groups also had a higher percentage of postmenopausal ($P = .0007$), black ($P = .0001$), and obese ($P < .0001$) patients compared with the nondiabetic group. The other potential prognostic factors were not found to be significantly different among the 3 groups. Aspirin was more frequently used in the nonmetformin group than in the metformin group ($P = .05$); however, there were no differences noted in the use of thiazolidinediones, statins, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers between the 2 diabetic groups.

Survival Estimates

The median follow-up for the patients who were still alive at the time of last follow-up was 62 months (range, 1 month-176 months). The Kaplan-Meier estimates of DMFS, RFS, and OS stratified by groups (metformin, nonmetformin, and nondiabetic) are shown in Figures 1A to 1C.

Of all the patients, 559 (39%) developed distant metastases: 18 (29%) in the metformin group, 26 (39%) in the nonmetformin group, and 515 (39%) in the nondiabetic group (Table 2). The 5-year DMFS estimates were 73%, 66%, and 60%, respectively, in the metformin group, the nonmetformin group, and the nondiabetic group ($P = .23$). When comparing patients who received metformin with patients who did not, the 5-year DMFS estimates were 60% versus 73% ($P = .09$). After adjustment for age, body weight, tumor size, lymph node status, nuclear grade, LVI, and type of adjuvant chemotherapy received, compared with the metformin group, patients who did not receive treatment with metformin (hazard ratio [HR], 1.63; 95% confidence interval [95% CI], 0.87-3.06 [$P = .13$]) and nondiabetic patients (HR, 1.62; 95% CI, 0.97-2.71 [$P = .06$]) tended to have a higher risk of developing distant metastases (Table 3).

Overall, there were 647 (44%) recurrences: 24 (38%) in the metformin group, 29 (43%) in the nonmetformin group, and 594 (45%) in the nondiabetic group. The estimated 5-year RFS rates were not different among the 3 groups (65% in the metformin group, 64% in the nonmetformin group, and 54% in the nondiabetic group; $P = .38$). After adjustment for age, body weight, tumor size, lymph node status, nuclear grade, LVI, and type of adjuvant chemotherapy received, there were no significant differences noted with regard to risk of disease recurrence between the nonmetformin (HR, 1.37; 95% CI, 0.78-2.40 [$P = .27$]) versus the metformin groups and the nondiabetic (HR, 1.36; 95% CI, 0.87-2.10 [$P = .17$]) versus the metformin groups.

On univariate analyses, older age ($>$ 50 years), smaller tumor size, absence of metastatic lymph nodes or LVI, undergoing BCS rather than mastectomy, and receiving adjuvant radiotherapy or anthracycline-based chemotherapy were found to be associated with higher DMFS and RFS rates. On multivariate analyses, older age, T1 to T2 status, N0 status, and negative LVI remained independent significant predictors of improved DMFS and RFS.

There were a total of 535 (37%) deaths: 20 (32%) in the metformin group, 23 (34%) in the nonmetformin group, and 492 (37%) in the nondiabetic group. The 5-year OS estimates were 67% in the metformin group, 69% in the nonmetformin group, and 66% in the nondiabetic group ($P = .58$). After adjustment for age, body weight, tumor size, lymph node status, nuclear grade, LVI, and type of adjuvant chemotherapy received, using the metformin group as the reference, the nonmetformin group (HR, 1.22; 95% CI, 0.66-2.28 [$P = .52$]) and the nondiabetic group (HR, 1.28; 95% CI, 0.79-2.08 [$P = .31$]) were each found to have a nonsignificantly increased risk of death. When comparing patients with diabetes who were receiving metformin versus those who were not, the P values (as determined by the log-rank test) were .29 for DMFS, .053 for RFS, and .80 for OS, respectively.

On univariate analyses, younger age ($\leq$ 50 years), T3 to T4 status, N1 to N3 status, positive LVI, and higher

Table 1. Patient Demographics and Baseline Clinical Characteristics by Study Group

	Metformin Group (N=63)		Nonmetformin Group (N=67)		Nondiabetic Group (N=1318)		
	No.	Percentage	No.	Percentage	No.	Percentage	*P*
Age at diagnosis, y							
Median (range)	53 (30-67)		51 (35-80)		48 (21-87)		
≤50	27	42.9	30	44.8	773	58.6	
>50	36	57.1	37	55.2	545	41.4	.005
Menopausal status							
Premenopausal	20	31.7	18	26.9	612	46.4	
Postmenopausal	43	68.3	49	73.1	706	53.6	.0007
Race							
White	24	38.1	30	44.8	939	71.2	
Black	22	34.9	24	35.8	185	14.0	
Hispanic	10	15.9	10	14.9	150	11.4	
Other	7	11.1	3	4.5	44	3.3	<.0001
Hemoglobin A1c level %[a]							
Median (range)	7.4 (5.4-12.0)		7.4 (5.6-9.5)		—		.78
Body weight, kg							
Median (range)	86 (58-140)		84 (50-130)		71 (35-192)		.14
Body mass index							
Median (range)	33.1 (23-52.5)		32.6 (18.5-67.1)		26.6 (16-75)		
Normal/underweight	6	10.7	11	17.5	457	37.5	
Overweight	13	23.2	10	15.9	394	32.3	
Obese	37	66.1	42	66.7	369	30.2	<.0001
Clinical T classification							
T1	26	41.3	28	41.8	661	50.2	
T2	31	49.2	35	52.2	578	43.9	
T3/4	6	9.5	4	6.0	79	6.0	.36
Clinical N classification							
N0	32	52.5	37	55.2	709	54.7	
N1	23	37.7	17	25.4	386	29.8	
N2	3	4.9	9	13.4	115	8.9	
N3	3	4.9	4	6.0	87	6.7	.58
Histology							
Ductal	56	88.9	63	94.0	1200	91.0	
Other	7	11.1	4	6.0	118	9.0	.58
Nuclear grade							
1 or 2	6	9.7	9	14.1	121	9.6	
3	56	90.3	55	85.9	1146	90.4	.49
LVI							
Negative	49	77.8	43	65.2	878	67.0	
Positive	14	22.2	23	34.8	433	33.0	.19
Chemotherapy type							
Anthracycline-based	19	30.2	30	44.8	548	41.6	
Taxane-based	3	4.8	1	1.5	45	3.4	
Anthracycline and taxane-based	30	47.6	29	43.3	612	46.4	
Other	11	17.5	7	10.4	113	8.6	.19
Ki-67							
Missing	29		22			—	
Low (<17)	5	14.7	7	15.6		—	
Medium (17-35)	9	26.5	9	20.0		—	
High (>35)	20	58.8	29	64.4		—	.79
Cardiac comorbidities						—	
None	58	92.1	55	82.1		—	
Coronary artery disease or congestive heart failure	5	7.9	12	17.9		—	.09
Cardiac risk factors						—	
None	16	25.4	14	20.9		—	
Dyslipidemia or hypertension	47	74.6	53	79.1		—	.54
Thiazolidinedione use						—	

(Continued)

Table 1. (Continued)

	Metformin Group (N=63)		Nonmetformin Group (N=67)		Nondiabetic Group (N=1318)		
	No.	Percentage	No.	Percentage	No.	Percentage	*P*
No	52	82.5	54	80.6		—	
Yes	11	17.5	13	19.4		—	.78
ACEI/ARB use						—	
No	30	47.6	37	55.2		—	
Yes	33	52.4	30	44.8		—	.39
Statin use						—	
No	34	54.0	34	50.7		—	
Yes	29	46.0	33	49.3		—	.71
Aspirin use						—	
No	47	74.6	39	58.2		—	
Yes	16	25.4	28	41.8		—	.05

Abbreviation: ACEI/ARBs, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; LVI, lymphovascular invasion.
[a] Based on available data from 35 patients in the metformin group and 28 patients in the nonmetformin group.

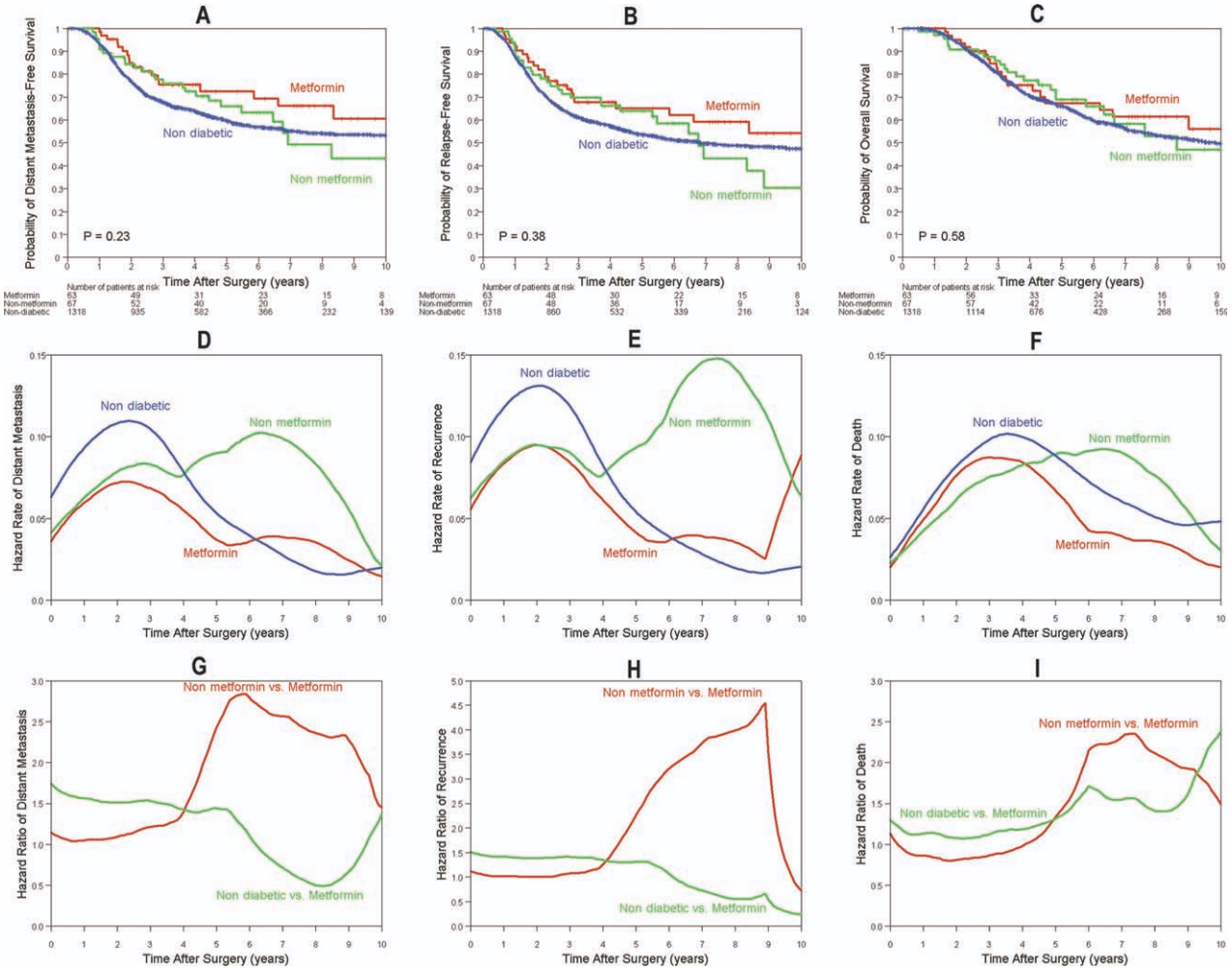

Figure 1. Kaplan-Meier estimates of (A) distant metastasis-free survival, (B) recurrence-free survival, and (C) overall survival are shown stratified by study groups (metformin, nonmetformin, and nondiabetic). Hazard functions for (D) distant metastasis, (E) disease recurrence, and (F) death are also shown stratified by groups. Hazard ratios of (G) distant metastasis, (H) disease recurrence, and (I) death for the nonmetformin versus metformin and nondiabetic versus metformin groups are shown among patients with triple-negative breast cancer.

Table 2. Five-Year Estimates for DMFS, RFS, and OS Rates Stratified According to Patient Demographics and Clinical Characteristics

			DMFS			RFS			OS	
	No. of Patients	No. of Events	5-Year Estimates (95% CI)	*P*	No. of Events	5-Year Estimates (95% CI)	*P*	No. of Events	5-Year Estimates (95% CI)	*P*[a]
All	1448	559	0.6 (0.58-0.63)		647	0.55 (0.52-0.57)		535	0.66 (0.64-0.69)	
Group										
Metformin	63	18	0.73 (0.58-0.83)		24	0.65 (0.51-0.76)	.38	20	0.67 (0.52-0.79)	
Nonmetformin	67	26	0.66 (0.52-0.77)		29	0.64 (0.5-0.75)		23	0.69 (0.55-0.79)	
Nondiabetic	1318	515	0.60 (0.57-0.62)	.23	594	0.54 (0.51-0.56)		492	0.66 (0.63-0.69)	.58
Metformin use										
No	1385	541	0.60 (0.57-0.63)		623	0.54 (0.51-0.57)		515	0.66 (0.64-0.69)	
Yes	63	18	0.73 (0.58-0.83)	.09	24	0.65 (0.51-0.76)	.20	20	0.67 (0.52-0.79)	.40
Daily dose of metformin										
0 mg	1385	541	0.60 (0.57-0.63)		623	0.54 (0.51-0.57)		515	0.66 (0.64-0.69)	
[500–1000] mg	49	14	0.72 (0.56-0.83)		18	0.66 (0.5-0.78)	.40	14	0.70 (0.53-0.82)	
>1000 mg	14	4	0.75 (0.4-0.91)	.24	6	0.63 (0.32-0.83)		6	0.56 (0.24-0.79)	.38
Diabetes										
No	1318	515	0.60 (0.57-0.62)		594	0.54 (0.51-0.56)	.21	492	0.66 (0.63-0.69)	
Yes	130	44	0.69 (0.59-0.77)	.15	53	0.65 (0.55-0.73)		43	0.68 (0.58-0.76)	.31
Age, y										
≤50	830	365	0.56 (0.52-0.59)	<.001	418	0.50 (0.46-0.53)	<.001	327	0.64 (0.61-0.68)	
>50	618	194	0.67 (0.63-0.71)		229	0.61 (0.57-0.65)		208	0.69 (0.65-0.73)	.14
Race										
Nonblack	1217	483	0.60 (0.57-0.63)		560	0.53 (0.5-0.56)		452	0.67 (0.64-0.69)	
Black	231	76	0.64 (0.57-0.71)	.18	87	0.61 (0.54-0.67)	.10	83	0.66 (0.59-0.73)	.58
Body mass index										
Normal/underweight	474	185	0.62 (0.57-0.66)		215	0.55 (0.50-0.60)		177	0.67 (0.62-0.71)	
Overweight	417	167	0.59 (0.54-0.64)		193	0.53 (0.48-0.58)		163	0.64 (0.58-0.69)	
Obese	448	168	0.61 (0.56-0.66)	.73	193	0.57 (0.51-0.61)	.65	152	0.71 (0.66-0.75)	.33
Clinical T classification										
T1-2	1359	505	0.62 (0.59-0.65)		586	0.56 (0.53-0.59)		482	0.68 (0.65-0.71)	
T3-4	89	54	0.40 (0.28-0.51)	<.001	61	0.32 (0.22-0.42)	<.001	53	0.42 (0.31-0.54)	<.001
Clinical N classification										
N0	778	226	0.70 (0.66-0.74)		272	0.64 (0.6-0.68)		211	0.77 (0.74-0.81)	
N1-3	647	324	0.49 (0.45-0.53)	<.001	364	0.43 (0.39-0.47)	<.001	313	0.55 (0.50-0.59)	<.001
Histology										
Ductal	1319	505	0.61 (0.58-0.63)		587	0.55 (0.52-0.57)		487	0.67 (0.64-0.69)	
Other	129	54	0.58 (0.47-0.66)	.25	60	0.54 (0.44-0.63)	.49	48	0.64 (0.54-0.73)	.39
Nuclear grade										
1 or 2	136	49	0.68 (0.58-0.75)		56	0.63 (0.53-0.71)		47	0.73 (0.63-0.80)	
3	1257	485	0.60 (0.57-0.63)	.21	564	0.54 (0.51-0.57)	.12	472	0.65 (0.62-0.68)	.05
LVI										
Negative	970	299	0.69 (0.66-0.72)		346	0.65 (0.61-0.68)		290	0.73 (0.7-0.76)	
Positive	470	256	0.43 (0.38-0.47)	<.001	296	0.34 (0.3-0.39)	<.001	243	0.52 (0.47-0.57)	<.001
Chemotherapy type										
Anthracycline-based	597	212	0.66 (0.62-0.7)		255	0.59 (0.55-0.63)		216	0.71 (0.67-0.75)	
Taxane-based	49	17	0.55 (0.36-0.71)		22	0.47 (0.29-0.63)		17	0.58 (0.38-0.74)	
Anthracycline and taxane-based	671	260	0.58 (0.54-0.62)		295	0.53 (0.49-0.57)		241	0.63 (0.59-0.67)	
Other	131	70	0.48 (0.38-0.57)	.001	75	0.43 (0.34-0.52)	.01	61	0.63 (0.54-0.72)	.006

Abbreviation: 95% CI, 95% confidence interval; DMFS, distant metastasis-free survival; LVI, lymphovascular invasion; OS, overall survival; RFS, recurrence-free survival.

[a] The log-rank *P* value tested the overall difference among comparison groups.

nuclear grade were found to be associated with an increased risk of death. In the multivariate model, the above noted prognostic features maintained their unfavorable impact with the exception of younger age. Within the diabetic groups, stratified analysis for body weight and daily metformin dose (0 mg vs 500-1000 mg vs > 1000 mg) did not reveal a significant difference in survival outcomes.

Table 3. Multivariate Cox Proportional Hazards Model for DMFS, RFS, and OS

	DMFS			RFS			OS		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Nonmetformin vs metformin	1.63	0.87-3.06	.13	1.37	0.78-2.40	.27	1.22	0.66-2.28	.52
Nondiabetic vs metformin	1.62	0.97-2.71	.06	1.36	0.87-2.10	.17	1.28	0.79-2.08	.31
Age: >50 y vs ≤50 y	0.70	0.58-0.85	.0002	0.71	0.60-0.84	<.0001	0.95	0.79-1.14	.57
Weight (continuous)	1.00	0.99-1.00	.60	1.00	0.99-1.00	.63	1.00	0.99-1.00	.43
Tumor classification: T3-4 vs T1-2	1.67	1.23-2.28	.001	1.59	1.19-2.13	.002	1.45	1.05-2.00	.023
Lymph node classification: N1-3 vs N0	1.77	1.46-2.16	<.0001	1.67	1.39-2.01	<.0001	1.71	1.39-2.09	<.0001
Nuclear grade: 3 vs 1/2	1.24	0.91-1.69	.18	1.26	0.94-1.68	.12	1.39	1.01-1.91	.043
LVI: positive vs negative	1.70	1.41-2.05	<.0001	1.81	1.52-2.16	<.0001	1.63	1.35-1.98	<.0001
Chemotherapy:									
T vs A	1.36	0.81-2.27	.25	1.23	0.77-1.97	.39	1.38	0.81-2.34	.23
AT vs A	0.98	0.80-1.20	.86	0.90	0.74-1.08	.25	0.99	0.81-1.22	.94
Other vs A	1.94	1.45-2.60	<.0001	1.72	1.30-2.27	.0001	1.55	1.14-2.10	.005

Abbreviations: 95% CI, 95% confidence interval; A, anthracycline-based regimens without a taxane; AT, anthracycline-based regimens with a taxane; DMFS, distant metastasis-free survival; HR, hazard ratio; LVI, lymphovascular invasion; OS, overall survival; RFS, recurrence-free survival; T, single-agent taxane without an anthracycline.

Figures 1D to 1I show kernel estimates of the hazard functions and HRs of distant metastasis, recurrence, and death, respectively. The 3-year and 5-year HRs for distant metastasis were 1.2 and 2.3, respectively, for the nonmetformin versus metformin groups and 1.5 and 1.4, respectively, for the nondiabetic versus metformin groups. The 3-year and 5-year HRs for disease recurrence were 1.1 and 2.3, respectively, for the nonmetformin versus metformin groups and 1.4 and 1.3, respectively, for the nondiabetic versus metformin groups. The 3-year and 5-year HRs for death were 0.9 and 1.3, respectively, for the nonmetformin versus metformin groups and 1.1 and 1.3, respectively, for the nondiabetic versus metformin groups.

DISCUSSION

Herein, we present the results of our single-institution study in a large cohort of women with TNBC, and assess the role of metformin during adjuvant chemotherapy in survival outcomes. The current study data suggest that concurrent use of metformin with adjuvant chemotherapy does not significantly impact survival outcomes in diabetic patients with TNBC; however, patients who do not take metformin and nondiabetic patients tend to have a higher risk of developing distant metastases.

In the current study cohort, diabetic patients were more likely to be black and obese. Although nondiabetic patients tended to be younger, they had a similar clinical stage of disease at the time of presentation compared with diabetic patients. Consistent with the findings of previous studies,[24,25] the histopathological tumor features were not different between the diabetic patients and nondiabetic patients. The choice of adjuvant chemotherapy did not differ between the 3 groups. We also evaluated the use of thiazolidinediones and other concomitant medications in the diabetic groups, because there is evidence that these agents (thiazolidinediones,[26] aspirin,[27,28] statins,[29,30] and angiotensin-converting enzyme inhibitors/angiotensin receptor blockers[31]) may affect tumorigenesis. Among these medications, only aspirin use was found to be significantly different between the 2 diabetic groups.

In contrast to previous studies,[25,32-34] our findings demonstrated that in this group of patients with TNBC, the presence of type 2 diabetes mellitus was not associated with lower survival outcomes, even when diabetic patients had other poor prognostic features including black race and obesity.[35-39] We could speculate that worse survival in diabetic patients is most likely ameliorated by strict glycemic control and therefore through the restoration of tumor sensitivity to chemotherapy and radiotherapy.

At the level of cell signaling, metformin is known to improve insulin resistance-associated hyperinsulinemia, mainly by affecting the insulin/IGF1 signaling pathway, which has been shown to induce cell cycle proliferation, tumor formation, and metastases.[40-42] Metformin also exerts direct inhibitory effects on the phosphatidylinositol 3-kinase (PI3K)/Akt/mTOR signaling pathway through activation of AMPK.[13,43-45] Consequently, metformin was associated with a decreased risk of breast cancer in patients with type 2 diabetes mellitus in various observational studies.[6,7] In addition, evidence suggests a lower

cancer-related mortality rate in diabetic patients who are treated with metformin compared with sulfonylurea preparations and, possibly, insulin therapy.[6,8,10,46,47]

It is not clear whether metformin use is predictive of improved long-term survival in patients with breast cancer. Epidemiological studies reported up to a 23% reduced risk of cancer-related mortality for metformin users compared with that for nonusers.[10,47] Jiralerspong et al reported a 3-fold greater pathologic complete response rate in diabetic patients with breast cancer who received metformin during neoadjuvant chemotherapy compared with those who did not (odds ratio, 2.95; 95% CI, 1.07-8.17 [$P = .04$]).[25] With a limited follow-up, the estimated 3-year RFS and OS rates tended to be better in the metformin group versus the nonmetformin group. However, metformin was not found to be an independent predictor of either RFS or OS after adjusting for diabetes status, body mass index, age, stage of disease, nuclear grade, ER/PR status, and taxane use. Although we did not observe a significant survival benefit with the concurrent use of metformin and adjuvant chemotherapy in our cohort of patients with TNBC, in the multivariate survival model, patients receiving metformin tended to have a reduced risk of developing distant metastasis compared with patients who were not receiving the drug ($P = .06$ when compared with nondiabetic patients). In addition, when the 2 diabetic groups were compared (metformin vs nonmetformin), there was a beneficial effect of metformin use noted in diabetic patients with regard to RFS ($P = .053$).

Metformin has been shown to reduce insulin levels by 22% in nondiabetic, hyperinsulinemic women with early stage breast cancer.[48] In clinical studies, elevated insulin levels have been associated with an increased rate of breast cancer recurrence and death.[49,50] Furthermore, there is recent evidence of the efficacy of nonpharmacologic interventions that reduce insulin resistance in affecting breast cancer outcomes. For example, in the Women's Intervention Nutrition Study,[24,51] lower dietary fat intake and weight loss in the intervention group correlated with lower rates of disease recurrence (9.8% vs 12.4%) and death (7.5% vs 18.1%) compared with the control group. In the subgroup analyses, dietary fat reduction had a greater effect on RFS in women with ER-negative breast tumors.

The known adverse prognostic factors such as younger age,[52,53] larger tumor size,[54] positive lymph node status, and LVI[55-57] were confirmed to be prognostic in the current analysis. We also demonstrated that patients treated with either single-agent taxane or non–anthracycline-containing/non–taxane-containing chemotherapy regimens had an increased risk of distant metastases, disease recurrence, and death. The differential influence of anthracycline-containing chemotherapy regimens on the outcome of patients with TNBC will require confirmation. Recently, Hirsch et al[58] demonstrated that combination therapy with metformin and doxorubicin reduces tumor mass and prevents disease recurrence much more effectively than either drug alone in a breast cancer xenograft mouse model. Moreover, selective inhibition of breast cancer stem cells by metformin appeared to have a dramatic effect on prolonging remission.[58] This interesting evidence provides the rationale for studying metformin with an anthracycline-based chemotherapy regimen as a new treatment for breast cancer.

Although previous studies have shown the potential efficacy of metformin as an antitumor agent in patients with breast cancer, these studies differ from the current work in that no account of the adjuvant breast cancer treatment was presented. However, several limitations must be considered when interpreting the results of the current study. It should be noted that our conclusions are mainly based on the subgroup of 63 metformin users and 1385 control patients, and therefore rely on a relatively small number of case and control patients. In addition, patient selection for individual treatment regimens may have influenced the differences in oncologic outcome. We did not have available data regarding the duration of diabetes, metformin use before the index date in the diabetic group, or metformin use in the nondiabetic group for other comorbid conditions such as polycystic ovary syndrome and nonalcoholic fatty liver disease. Another limitation is that the level of surveillance and detection of nonfatal outcomes in particular might vary according to diabetes status. We also could not add the comorbidity score as a potential adjustment variable because we did not have enough information regarding the severity of comorbid conditions. Hence, the potential impact of these confounding factors on outcome need to be acknowledged.

In conclusion, the results of the current retrospective analysis suggest that metformin use during adjuvant therapy was not associated with improved survival outcomes in diabetic patients with TNBC. However, there was a trend toward a decrease in the risk of developing distant metastasis in diabetic patients receiving metformin compared with nondiabetic patients. In light of the evidence that the majority of breast cancer deaths result from

distant metastatic recurrence, these findings deserve to be tested with additional prospective studies. Currently, the impact of metformin on survival outcomes in patients treated with adjuvant breast cancer therapy is being investigated in a phase 3 randomized trial conducted by the National Cancer Institute of Canada Clinical Trials Group.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

REFERENCES

1. Richardson LC, Pollack LA. Therapy insight: influence of type 2 diabetes on the development, treatment and outcomes of cancer. *Nat Clin Pract Oncol.* 2005;2:48-53.
2. Frasca F, Pandini G, Sciacca L, et al. The role of insulin receptors and IGF-I receptors in cancer and other diseases. *Arch Physiol Biochem.* 2008;114:23-37.
3. Lipscombe LL, Goodwin PJ, Zinman B, McLaughlin JR, Hux JE. Increased prevalence of prior breast cancer in women with newly diagnosed diabetes. *Breast Cancer Res Treat.* 2006;98:303-309.
4. Wolf I, Sadetzki S, Gluck I, et al. Association between diabetes mellitus and adverse characteristics of breast cancer at presentation. *Eur J Cancer.* 2006;42:1077-1082.
5. Hjartaker A, Langseth H, Weiderpass E. Obesity and diabetes epidemics: cancer repercussions. *Adv Exp Med Biol.* 2008;630:72-93.
6. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. *BMJ.* 2005;330:1304-1305.
7. Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. *Diabetes Care.* 2009;32:1620-1625.
8. Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia.* 2009;52:1766-1777.
9. Bodmer M, Meier C, Krahenbuhl S, Jick SS, Meier CR. Long-term metformin use is associated with decreased risk of breast cancer. *Diabetes Care.* 2010;33:1304-1308.
10. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin: response to Farooki and Schneider. *Diabetes Care.* 2006;29:1990-1991.
11. Berstein LM. Clinical usage of hypolipidemic and antidiabetic drugs in the prevention and treatment of cancer. *Cancer Lett.* 2005;224:203-212.
12. Hadad SM, Fleming S, Thompson AM. Targeting AMPK: a new therapeutic opportunity in breast cancer. *Crit Rev Oncol Hematol.* 2008;67:1-7.
13. Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. *Cancer Res.* 2007;67:10804-10812.
14. Rice S, Pellatt L, Ramanathan K, Whitehead SA, Mason HD. Metformin inhibits aromatase via an extracellular signal-regulated kinase-mediated pathway. *Endocrinology.* 2009;150:4794-4801.
15. Berstein LM. Metformin, insulin, breast cancer and more... *Future Oncol.* 2009;5:309-312.
16. Brown KA, Hunger NI, Docanto M, Simpson ER. Metformin inhibits aromatase expression in human breast adipose stromal cells via stimulation of AMP-activated protein kinase. *Breast Cancer Res Treat.* 2010;123:591-596.
17. Alimova IN, Liu B, Fan Z, et al. Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro. *Cell Cycle.* 2009;8:909-915.
18. Liu B, Fan Z, Edgerton SM, et al. Metformin induces unique biological and molecular responses in triple negative breast cancer cells. *Cell Cycle.* 2009;8:2031-2040.
19. Ben Sahra I, Laurent K, Loubat A, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. *Oncogene.* 2008;27:3576-3586.
20. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, eds. AJCC Cancer Staging Manual and Handbook. 7th ed. New York: Springer; 2010.
21. The World Health Organization Histological Typing of Breast Tumors-Second Edition. The World Organization. *Am J Clin Pathol.* 1982;78:806-816.
22. Black MM, Speer FD. Nuclear structure in cancer tissues. *Surg Gynecol Obstet.* 1957;105:97-102.
23. Hess KR, Serachitopol DM, Brown BW. Hazard function estimators: a simulation study. *Stat Med.* 1999;18:3075-3088.
24. Chlebowski RT, Blackburn GL, Thomson CA, et al. Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. *J Natl Cancer Inst.* 2006;98:1767-1776.
25. Jiralerspong S, Palla SL, Giordano SH, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. *J Clin Oncol.* 2009;27:3297-3302.
26. Galli A, Mello T, Ceni E, Surrenti E, Surrenti C. The potential of antidiabetic thiazolidinediones for anticancer therapy. *Expert Opin Investig Drugs.* 2006;15:1039-1049.
27. Ararat E, Sahin I, Altundag K. Aspirin intake may prevent metastasis in patients with triple-negative breast cancer [published online ahead of print July 29, 2010]. *Med Oncol.*
28. Holmes MD, Chen WY, Li L, Hertzmark E, Spiegelman D, Hankinson SE. Aspirin intake and survival after breast cancer. *J Clin Oncol.* 2010;28:1467-1472.
29. Ghosh-Choudhury N, Mandal CC, Ghosh-Choudhury N, Ghosh Choudhury G. Simvastatin induces derepression of PTEN expression via NFkappaB to inhibit breast cancer cell growth. *Cell Signal.* 2010;22:749-758.
30. Garwood ER, Kumar AS, Baehner FL, et al. Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer. *Breast Cancer Res Treat.* 2010;119:137-144.

31. Molteni A, Heffelfinger S, Moulder JE, Uhal B, Castellani WJ. Potential deployment of angiotensin I converting enzyme inhibitors and of angiotensin II type 1 and type 2 receptor blockers in cancer chemotherapy. *Anticancer Agents Med Chem.* 2006;6:451-460.
32. Yancik R, Wesley MN, Ries LA, Havlik RJ, Edwards BK, Yates JW. Effect of age and comorbidity in postmenopausal breast cancer patients aged 55 years and older. *JAMA.* 2001;285:885-892.
33. Verlato G, Zoppini G, Bonora E, Muggeo M. Mortality from site-specific malignancies in type 2 diabetic patients from Verona. *Diabetes Care.* 2003;26:1047-1051.
34. Coughlin SS Calle EE, Teras LR, Petrelli J, Thun MJ. Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. *Am J Epidemiol.* 2004;159:1160-1167.
35. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA.* 2006;295:2492-2502.
36. Woodward WA, Huang EH, McNeese MD, et al. African-American race is associated with a poorer overall survival rate for breast cancer patients treated with mastectomy and doxorubicin-based chemotherapy. *Cancer.* 2006;107:2662-2668.
37. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet.* 2008;371:569-578.
38. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med.* 2003;348:1625-1638.
39. Fleming ST, Rastogi A, Dmitrienko A, Johnson KD. A comprehensive prognostic index to predict survival based on multiple comorbidities: a focus on breast cancer. *Med Care.* 1999;37:601-614.
40. Baserga R, Peruzzi F, Reiss K. The IGF-1 receptor in cancer biology. *Int J Cancer.* 2003;107:873-877.
41. Lopez T, Hanahan D. Elevated levels of IGF-1 receptor convey invasive and metastatic capability in a mouse model of pancreatic islet tumorigenesis. *Cancer Cell.* 2002;1:339-353.
42. Jones RA, Campbell CI, Gunther EJ, et al. Transgenic overexpression of IGF-IR disrupts mammary ductal morphogenesis and induces tumor formation. *Oncogene.* 2007;26:1636-1644.
43. Gonzalez-Angulo AM, Meric-Bernstam F. Metformin: a therapeutic opportunity in breast cancer. *Clin Cancer Res.* 2010;16:1695-1700.
44. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest.* 2001;108:1167-1174.
45. Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. *Circ Res.* 2007;100:328-341.
46. Goodwin PJ, Pritchard KI, Ennis M, Clemons M, Graham M, Fantus IG. Insulin-lowering effects of metformin in women with early breast cancer. *Clin Breast Cancer.* 2008;8:501-505.
47. Landman GW, Kleefstra N, van Hateren KJ, Groenier KH, Gans RO, Bilo HJ. Metformin associated with lower cancer mortality in type 2 diabetes: ZODIAC-16. *Diabetes Care.* 2010;33:322-326.
48. Goodwin PJ, Pritchard KI, Ennis M, et al. Metformin lowers fasting insulin levels in nondiabetic hyperinsulinemic early stage breast cancer patients. *Breast Cancer Res Treat.* 2006(Suppl 1);100:109 Abst 2085.
49. Goodwin PJ, Ennis M, Pritchard KI, et al. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. *J Clin Oncol.* 2002;20:42-51.
50. Pollak MN, Chapman JW, Shepherd L, et al. Insulin resistance, estimated by serum C-peptide level, is associated with reduced event-free survival for postmenopausal women in NCIC CTG MA. 14 adjuvant breast cancer trial. *J Clin Oncol.* 2006 ASCO Annual Meeting Proceedings Part 1;24(June 20 Suppl):524.
51. Chlebowski RT, Blackburn GL, Hoy MK, et al. Survival analyses from the Women's Intervention Nutrition Study (WINS) evaluating dietary fat reduction and breast cancer outcome. *J Clin Oncol.* 2008;26(12 suppl):26 (May 20 Suppl): Abst 522.
52. Livasy CA, Karaca G, Nanda R, et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol.* 2006;19:264-271.
53. Rodriguez-Pinilla SM, Sarrio D, Honrado E, et al. Prognostic significance of basal-like phenotype and fascin expression in node-negative invasive breast carcinomas. *Clin Cancer Res.* 2006;12:1533-1539.
54. Dawood S, Broglio K, Kau SW, et al. Triple receptor-negative breast cancer: the effect of race on response to primary systemic treatment and survival outcomes. *J Clin Oncol.* 2009;27:220-226.
55. Dent R, Hanna WM, Trudeau M, Rawlinson E, Sun P, Narod SA. Time to disease recurrence in basal-type breast cancers: effects of tumor size and lymph node status. *Cancer.* 2009;115:4917-4923.
56. Solin LJ, Hwang WT, Vapiwala N. Outcome after breast conservation treatment with radiation for women with triple-negative early-stage invasive breast carcinoma. *Clin Breast Cancer.* 2009;9:96-100.
57. Cheang MC, Voduc D, Bajdik C, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res.* 2008;14:1368-1376.
58. Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. *Cancer Res.* 2009;69:7507-7511.