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14. ABSTRACT

In the first year of his predoctoral award, Thomas Ahern completed all coursework and qualifying examinations required for entry into the senior phase of doctoral study for the Doctor of Science degree in epidemiology at Boston University. Mr. Ahern submitted a dissertation research proposal to the Epidemiology Doctoral Committee in May 2008; the proposal was formally approved in October 2008. Mr. Ahern completed and published one of the three studies of his dissertation research in December 2008, an investigation of the effects of cardiac glycoside treatment on breast cancer risk. Mr. Ahern has supervised the ongoing collection and processing of biological samples required for his second and third dissertation projects. These studies are on track for completion in 2010. Meanwhile Mr. Ahern has published several other breast cancer research papers as a supplement to his training program.

15. SUBJECT TERMS
Breast cancer, cardiac glycosides, tamoxifen, UDP-glucuronosyl transferases, p21-activated kinase 1, epidemiology

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Introduction:

Thomas Ahern is a candidate for the Doctor of Science (D.Sc.) degree in epidemiology at the Boston University School of Public Health. Mr. Ahern's pre-doctoral training program includes advanced coursework in epidemiology and biostatistics, collaboration on an international, multidisciplinary research team to study molecular, genetic, and pharmaceutical determinants of breast cancer outcomes, teaching responsibilities in epidemiologic methods courses, and a dissertation project focused on the impact of prescription drugs on the risk of breast cancer incidence and recurrence. This annual report summarizes Mr. Ahern's accomplishments since receipt of his CDMRP pre-doctoral award in April 2008, with special emphasis on progress made during the second year of the award.

Body:

Component 1: Junior Doctoral Study

Thomas Ahern completed all required coursework for his doctoral degree in May of 2008, with a cumulative grade point average of 3.8. Just prior to this, he successfully passed both required sections of the doctoral qualifying examination for epidemiology (epidemiology section: summer 2007; biostatistics section: winter 2007). These achievements enabled Mr. Ahern to embark on his dissertation research, and marked the completion of Tasks 1 and 2 under the heading of "Junior Doctoral Study" in the Statement of Work (please note that a revised SOW was approved by USAMRAA in February 2010).

Component 2: Cardiac Glycoside Treatment and Breast Cancer Risk (first dissertation study)

Mr. Ahern's three dissertation studies utilize the nationwide medical registries of Denmark to evaluate associations between prescription drugs and breast cancer outcomes. The first dissertation study evaluated the association between cardiac glycoside therapy (e.g., digoxin) and the risk of newly diagnosed breast cancer. Tasks for this study appear in the "Cardiac Glycoside Treatment and Breast Cancer Risk" component of the SOW. In 2008, Mr. Ahern enumerated a case-control data set of women with a new diagnosis of breast cancer and age-matched cancer-free controls (Task 1). Mr. Ahern then linked this roster of subjects to Danish prescription drug registries in order to characterize cardiac glycoside exposure in the two groups of women (Task 2). Using this data set, which also contained information on important confounding and modifying covariates, Mr. Ahern carried the statistical analyses necessary to estimate the relative risk of breast cancer comparing women who were exposed to cardiac glycosides with women who were not exposed to cardiac glycosides, accounting for important covariates. Mr. Ahern then prepared a manuscript for this study with guidance from his thesis committee (the co-authors), marking the completion of Task 3 of this component of

the SOW. The manuscript was published in December 2008 in the open-access online journal *Breast Cancer Research*.[1] The paper was featured in national print media through a syndicated article by *Reuters Health.* According to the journal's tracking system, at the time of this writing, the manuscript has been downloaded more than 2,900 times.

Major findings from this work indicate an increased risk of breast cancer incidence following any treatment with digoxin (adjusted breast cancer odds ratio = 1.30; 95% CI: 1.14, 1.48). This association grew somewhat stronger as the duration of digoxin treatment increased (adjusted breast cancer odds ratio for ≥ 7 years of treatment = 1.39; 95% CI: 1.10, 1.74). A copy of the manuscript appears in the appendix.

Component 3: Treatment with Vitamin K Antagonists and Cancer Risk (second dissertation study)

Mr. Ahern's second dissertation study evaluates the association between treatment with vitamin K antagonist anticoagulants (VKAs) and the incidence of breast and other site-specific cancers. Tasks for this study appear in the "Treatment with Vitamin K Antagonists and Cancer Risk" component of the SOW. This study utilizes heart valve transplant as a proxy for treatment with a VKA, since actual prescription data were not automatically collected in Denmark until the mid-1990s. The strength of heart valve transplant in predicting VKA treatment was to be estimated in a validation subset, allowing for correction of bias due to imperfect exposure measurement (via the heart valve proxy) using modern probabilistic sensitivity analysis techniques.

In October 2009, Mr. Ahern traveled to Aarhus University in Aarhus, Denmark, to complete the first three tasks of this study. Using the Danish National Registry of Patients, Mr. Ahern constructed a cohort comprised of (1) all Danes who received a heart valve transplant between 01 January 1977 and 31 December 2006, and who had no prior cancer diagnosis as of their transplant date, and (2) an age-and sex-matched reference group of Danes who had not received a heart valve transplant and who were also without a prior cancer diagnosis on the same date of the matched heart valve recipient's transplant date (Task 1). Mr. Ahern then linked the cohort to the Danish Cancer Registry to ascertain the first incident cancer (if any) for all subjects (Task 2). The vital status of cancer-free subjects was ascertained by linkage to the Danish Civil Registry (Task 2).

Using the subset of cohort members whose follow-up time occurred within the period of prescription data coverage, Mr. Ahern linked to county-specific prescription databases and conducted a validation study to measure the accuracy with which heart valve transplant history measures actual exposure to a VKA. From these validation data, Mr. Ahern calculated estimates of the positive and negative predictive values (PPV and NPV, respectively) for the heart valve proxy variable (Task 3). Associations between known VKA exposure and

incidence of site-specific cancers were calculated for the subjects in the validation subset; associations between the heart valve proxy and incidence of site-specific cancers were calculated for subjects who were not in the validation subset Task 4a). For the latter vector of associations, Mr. Ahern implemented a probabilistic bias analysis algorithm to adjust the estimates based on the positive and negative predictive values calculated from the validation subset. The corrected data and validation data were then combined to yield single estimates of the associations between VKA treatment and incidence of the various site-specific cancers (Task 4b). Mr. Ahern is presently preparing a manuscript based on this work (Task 4).

According to analyses completed at the time of this writing, results of the study show that heart valve transplant is a powerful proxy variable for receipt of VKA therapy (PPV = 97% NPV = 91%). The overall pattern of ranked, corrected associations between heart valve transplant and incident site-specific cancers indicates no convincing association between VKA therapy and cancer incidence at any anatomic site (Figure 1), despite the fact that some associations appear modestly elevated, with accompanying 95% simulation intervals that exclude the null.

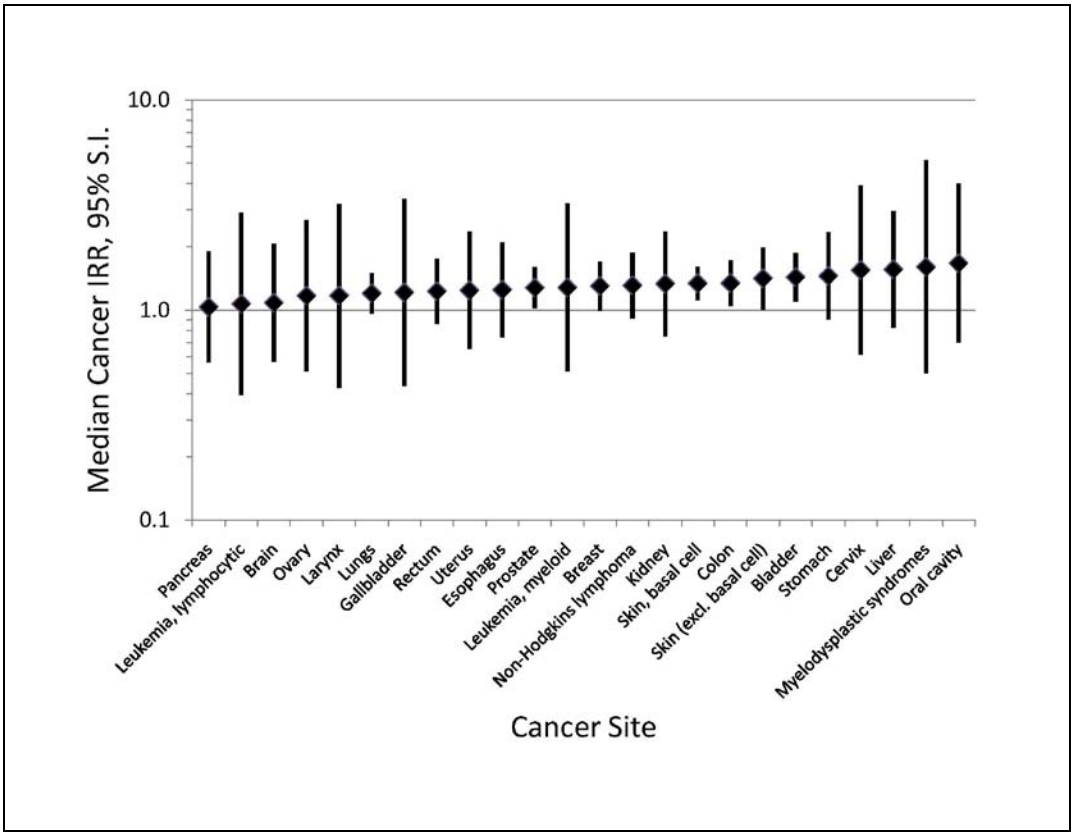

Figure 1: Associations between heart valve treatment and site-specific cancer incidence, corrected for the predictive ability of heart valve replacement for receipt of vitamin K antagonist therapy

Component 4: Use of Statin Medications and Risk of Breast Cancer Recurrence (third dissertation study)

In order to receive the data necessary to conduct this study, Mr. Ahern applied for and received approval from two Danish registry oversight boards: the Danish Breast Cancer Cooperative Group (DBCG; approval granted in November 2009) and Statistics Denmark, which oversees national prescription drug data (approval granted February 2010), which marks completion of Task 1 of this component. Mr. Ahern's thesis committee chair, Dr. Timothy Lash, was recently awarded a sum of money by the Danish Clinical Institute sufficient to support creation of the data set by statisticians at the DBCG and Statistics Denmark. Completion of the remaining tasks of this component will occupy the spring and summer months of 2010, leading up to Mr. Ahern's thesis defense.

Component 5: Senior Doctoral Study

Mr. Ahern plans to submit one or more abstracts for the 2010 San Antonio Breast Cancer Symposium, and continues to present his work locally to the Boston University community (Tasks 1 and 3).

Mr. Ahern has carried out a number of teaching responsibilities during his training program. In the fall semester of 2007, Mr. Ahern served as a teaching assistant to Dr. Timothy Lash for his course "Modern Epidemiology" (EP854); in the spring of 2008, Mr. Ahern was a teaching assistant to Drs. Matthew Fox and Barbara Mahon for their course "Infectious Disease Epidemiology" (EP755); in the fall of 2008 Mr. Ahern was a teaching assistant to Dr. Daniel Brooks for his course "Epidemiologic Methods" (EP712). In the fall semester of 2009, Mr. Ahern was selected to serve as co-instructor for two epidemiologic methods courses offered by his department: "Introduction to Epidemiology" (EP711), which enrolled approximately 130 master's-level students; and "Design and Conduct of Cohort Studies" (EP857), a seminar-style course comprised mostly of epidemiology doctoral students. Mr. Ahern has also delivered four guest lectures in other epidemiologic methods courses, with topics ranging from observational study design to Bayesian statistical analysis (Task 4).

Additional accomplishments:

Beyond the aims detailed in the SOW, Mr. Ahern has conducted and published several breast cancer studies which have augmented his training program. The first of these characterized the temporal trend in breast-conserving surgery use by Danish surgical oncologists and their patients, in relation to the publication of three major trials demonstrating survival equivalency of the two procedures; this study was published in the *European Journal of Epidemiology*.[2] The second of these studies explored the effect of comorbid disease on all-cause mortality in breast cancer survivors, where comorbidity status was either assessed at breast cancer diagnosis or updated repeatedly throughout follow-up; this study was published in the health services

journal, *Medical Care*.[3] The third study explored the association between lifetime exposure to tobacco smoke and the incidence of breast cancer; this manuscript was published in *Cancer Causes and Control*.[4] The fourth study demonstrated the hazards of combining categories of comorbidity indices when making inferences in clinical epidemiology studies, using a cohort of breast cancer patients as an example; this study was published in the new, open-access online journal *Clinical Epidemiology*.[5] The fifth study evaluated the interaction between tamoxifen and drugs that inhibit CYP2D6, the enzyme responsible for generating physiologically active tamoxifen metabolites, to see whether CYP2D6-inhibiting co-prescriptions reduced tamoxifen's effectiveness, therefore elevating breast cancer recurrence rates among women with estrogen receptor-positive breast tumors undergoing adjuvant tamoxifen therapy; this study was published in *Cancer Epidemiology, Biomarkers and Prevention*.[6] In addition to these first-authored papers, Mr. Ahern co-authored two other original research articles with Dr. Timothy Lash and his colleagues in Denmark, which examined reduction of tamoxifen effectiveness by SSRI antidepressants.[7, 8] Mr. Ahern also served as statistician for an abstract submitted for the 2010 meeting of the American Society for Clinical Oncology, which reports differences in breast tumor characteristics between breast cancer patients from multiple ethnicities.[9] Copies of published manuscripts resulting from these efforts appear in the appendix.

Mr. Ahern has also completed a number of invited peer reviews of breast cancer manuscripts submitted to leading journals, including *Breast Cancer Research and Treatment, British Medical Journal*, *American Journal of Epidemiology*, *The Breast*, *European Journal of Epidemiology*, and *Journal of Clinical Epidemiology*.

Mr. Ahern was invited by the chair of the epidemiology department to co-instruct two epidemiologic methods courses in the Fall semester of 2009: EP711, "Introduction to Epidemiology", was co-taught with Drs. Elizabeth Lawler and Jaimie Gradus. EP857, "Design and Conduct of Cohort Studies", was co-taught with Dr. Lauren Wise.

Key Program Accomplishments:

- Successfully passed required qualifying examination (epidemiology and biostatistics sections) for D.Sc. degree in epidemiology, Fall 2007
- Completed required coursework for D.Sc. degree in epidemiology, May 2008.
- First dissertation study (exploring association between digoxin treatment and breast cancer risk) conducted and published.[1]
- Co-authored two original papers on the effect of SSRI antidepressant co-prescription on tamoxifen effectiveness.[7, 8]
- Collaborated with researchers at Boston University to study breast cancer tumor factors according to ethnicity; abstract submitted for the 2010 meeting of the American Society for Clinical Oncology.[9]
- Conducted and published a case-control study of the effect of lifetime tobacco smoke exposure on the incidence of breast cancer.[4]
- Conducted a published a case-control study of the interaction between CYP2D6-inhibiting prescription drugs and tamoxifen, and whether such interaction diminishes tamoxifen effectiveness.[6]
- Published an illustration of the harmful effects of combining upper categories of a comorbidity index in clinical epidemiology studies by using a cohort of older breast cancer survivors as an example population.[5]
- Conducted and published a population-based study to report the trend in the uptake of breast-conserving surgery over time in Denmark.[2]
- Conducted and published a cohort study to evaluate whether time-varying assessment of comorbidity status increased the association between comorbidity and mortality in breast cancer survivors.[3]
- Co-authored two letters to journal editors with Dr. Lash and members of the Danish collaboration.[10, 11]
- Served as a teaching assistant for EP854, “Modern Epidemiology” (Professor Timothy Lash); Fall 2006 and Fall 2007.
- Served as a teaching assistant for EP755, “Infectious Disease Epidemiology” (Professors Barbara Mahon, Matthew Fox and Robert Horsburgh); Spring 2007 and Spring 2008.
- Served as a teaching assistant for EP712, “Epidemiologic Methods” (Professor Dan Brooks); Fall 2008.
- Delivered a guest lecture on the conduct of case-control studies to EP711, “Introduction to Epidemiology” (Professor Elizabeth Lawler and Ryan Ferguson); Fall 2008.
- Orally presented results from first dissertation study (digoxin and breast cancer) at the Epidemiology Department’s Research in Progress seminar; Fall 2008.
- Gave a poster presentation of a study of acquired comorbidity and mortality among breast cancer patients at Boston University’s Science and Engineering Research Symposium; Spring 2008.

Reportable Outcomes:

- Publication of 8 original breast cancer research papers, one of which applies toward the research requirements for the D.Sc. degree in epidemiology.[1-8] Copies of published manuscripts appear in the appendix of this report.

Conclusion:

Mr. Ahern has made substantial progress toward the completion of the D.Sc. degree in epidemiology in the first two years of his CDMRP pre-doctoral award. For part of his dissertation, he published the largest study to date of the effect of cardiac glycoside treatment on breast cancer risk,[1] in addition to eight breast cancer studies beyond those that form his dissertation research.[2-8] He has co-instructed two major epidemiologic methods courses at the Boston University School of Public Health, delivered a variety of guest lectures to other courses, and has served as a peer reviewer for a number of esteemed epidemiologic and medical journals. Mr. Ahern's contributions to the breast cancer research field, enabled by his CDMRP pre-doctoral award, provide new knowledge to scientists and clinicians in the field, and will help to advance breast cancer risk assessment and treatment technology.

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Appendix:

The following pages contain copies of manuscripts authored or co-authored by Mr. Ahern since the beginning of his pre-doctoral award, and an updated copy of his *curriculum vitae*.

Acta Oncologica, 2010; Early Online, 1–8

informa
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ORIGINAL ARTICLE

Breast cancer recurrence risk related to concurrent use of SSRI antidepressants and tamoxifen

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Abstract

Background. Up to one-quarter of breast cancer patients suffer clinically significant depression in the year after diagnosis, which may respond to intervention. About half may be prescribed a psychotropic medication, such as a selective serotonin reuptake inhibitor (SSRI), while completing breast cancer therapy. Cytochrome P-450 2D6 (*CYP2D6*) metabolizes SSRIs and also metabolizes tamoxifen to more active forms. Therefore, concurrent use of SSRIs may reduce tamoxifen's effectiveness at preventing breast cancer recurrence. The SSRI citalopram has limited potency to inhibit *CYP2D6* activity, so has been recommended for breast cancer patients taking tamoxifen. This study provides epidemiologic evidence to support this recommendation. *Material and methods*. We conducted a case-control study of breast cancer recurrence nested in the population of female residents of Denmark who were diagnosed with non-metastatic estrogen-receptor positive breast cancers between 1994 and 2001 and who took tamoxifen for at least one year. We ascertained complete prescription histories by linking cases' and controls' civil registration numbers to the Danish national prescription registry. We estimated the association between SSRI use while taking tamoxifen and risk of recurrent breast cancer. *Results.* About the same proportion of recurrent cases (37 of 366) and matched controls (35 of 366) received at least one prescription for citalopram or its s-stereoisomer while taking tamoxifen (adjusted odds ratio=1.1, 95% confidence interval=0.7, 1.7). Breast cancer patients taking other SSRIs were also at no increased risk of recurrence (adjusted odds ratio=0.9, 95% confidence interval=0.5, 1.8). *Discussion*. Breast cancer patients with indications for an SSRI may be prescribed citalopram – and possibly other SSRI – without adversely affecting the outcome of adjuvant therapy with tamoxifen.

Key Words: *Breast neoplasms, pharmacology and therapeutic use; tamoxifen, antagonists and inhibitors; serotonin uptake inhibitors; cytochrome P-450 2D6*

Almost all newly diagnosed breast cancer patients experience normal distress [1]. Up to one-quarter, however, suffer clinically significant depression in the year after diagnosis, which may respond to interventions [2]. Although no study has yet examined the effectiveness of psychotherapy and psychiatric drug therapy in cancer patients [2], the prevalence of prescriptions for psychotropic drugs among patients treated for breast cancer is high. For example, about half of the patients in a breast cancer waiting-room sample had received psychotropic medication during their breast cancer treatment [3]. One class of psychotropic medications, selective serotonin reuptake inhibitors (SSRI), may reduce both depressive symptoms and menopausal symptoms [4,5].

SSRIs are metabolized by cytochrome P450 2D6 (*CYP2D6*) [6], as is the selective estrogen-receptor modulator tamoxifen [7]. Tamoxifen reduces the risk of breast cancer recurrence by about half in patients with estrogen-receptor positive tumors [8].

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Tamoxifen's two 4-hydroxylated metabolites have the highest binding affinity for the estrogen receptor, and are the most important modulators of the estrogen receptor in the tamoxifen pathway [9,10]. Because both tamoxifen and SSRIs are metabolized by *CYP2D6*, SSRI inhibition of *CYP2D6* activity could reduce tamoxifen's prevention of breast cancer recurrence. Citalopram is among the least potent SSRI inhibitors of *CYP2D6* activity [6,11], which led Henry et al. to recommend citalopram (or similar low-inhibiting venlafaxine) for treatment of breast cancer patients taking tamoxifen [12]. Aside from a preliminary report from this research group [13], no clinical epidemiologic evidence has shown that these SSRI do not interfere with tamoxifen's effect on breast cancer recurrence risk.

We previously reported that Danish breast cancer patients with estrogen-receptor positive tumors who were treated with tamoxifen had no higher rate of recurrence if they were simultaneously taking the SSRI citalopram or its s-stereoisomer than if they were not [13]. This initial study was limited to four counties with local prescription registries. We have now extended the study to eight counties and use Danish national prescription data to ascertain exposure to SSRI and to control for exposure to a wide range of medications. The current study has substantially improved precision and has allowed more complete investigation of other *CYP2D6*-inhibiting SSRI medications.

Material and methods

The study was approved by the Boston University Medical Campus Institutional Review Board and the Regional Committee on Biomedical Research Ethics of Aarhus County, Denmark. Because the data are housed in medical registries, individual informed consent was not obtained.

Study population

The source population included female residents of eight Danish counties (Funen, South Jutland, Ribe, Vejle, Ringkøbing, Aarhus, Viborg, and North Jutland) 35–69 years old at diagnosis of stage I, II or III primary breast cancer between 1994–2001 and who were reported to the Danish Breast Cancer Cooperative Group (DBCG; [14]). We divided the source population into three groups: (a) ER+/TAM+ – estrogen-receptor positive and treated with tamoxifen for at least one year without recurrence in that year, (b) ER−/TAM− – estrogen-receptor negative, not treated with tamoxifen, and survived recurrence-free at least one year, and (c) group III women – all others, including patients who recurred in the first year, ER+ patients who did not receive tamoxifen, and ER− patients who did receive tamoxifen, all of whom were excluded from this analysis. Estrogen receptor expression was assayed at diagnosing hospitals by standard DBCG protocols. Clinical assay of estrogen receptor expression in pathology laboratories has shown high concordance with centralized testing in similar settings [15]. ER+/TAM+ women were assigned to tamoxifen therapy protocols of one year, two years, or five years, depending on the guideline current in Denmark when they were diagnosed [16]. Many of the women assigned to tamoxifen protocols shorter than five years took tamoxifen for much longer (unpublished validation data). Follow-up time began one year after breast cancer diagnosis and continued until the date of the first of breast cancer recurrence, death from any cause, loss to follow-up (e.g., emigration), 10 years of follow-up, or September 1, 2006.

Cases were women with local or distant breast cancer recurrence during their follow-up time. We used the DBCG definition of breast cancer recurrence as any type of breast cancer subsequent to the initial course of therapy. Using risk-set sampling, we matched one control to each case on (a) group membership (ER+/TAM+ or ER−/TAM−), (b) menopausal status at diagnosis (premenopausal or postmenopausal), (c) date of breast cancer surgery (caliper matched +/− twelve months), (d) county of residence at time of diagnosis, and (e) stage at diagnosis (stage I, II, or III). Controls were free of breast cancer recurrence at the same duration of post-surgery follow-up as their matched case. It was not possible to match controls to cases on duration of tamoxifen therapy, but the calendar time matching induced by risk-set sampling afforded good balance between cases and controls with regard to the duration of assigned tamoxifen protocol.

Data collection

We used the Danish civil registration number (CPR) assigned to all Danish citizens and residents to link data sets. We collected demographic information (age, menopausal status, and hospital of diagnosis), tumor characteristics (UICC stage, histologic grade, and estrogen-receptor expression), and therapy characteristics (primary surgical tumor management, receipt of radiation therapy, receipt of chemotherapy, and receipt of tamoxifen therapy) from the DBCG database. We collected data on receipt of citalopram prescriptions, prescriptions for other SSRIs, and prescriptions for other potential *CYP2D6* inhibitors by linking the CPR numbers of cases and controls to the prescription database maintained by Statistics Denmark as a component of the Danish national health care system.

Analytic variables

Prescription status. Prescription medications were coded by the Anatomical Therapeutic Chemical (ATC) classification system [17]. We defined SSRIs as all those classified in ATC group N06AB. We combined prescriptions for citalopram with prescriptions for its s-enantiomer escitalopram because escitalopram inhibits *CYP2D6* activity similarly to citalopram *in vitro* [18] and both have been shown to inhibit *CYP2D6* activity *in vivo* [11,19]. We classified cases and controls as those with no record of a citalopram prescription during their follow-up time (never citalopram) and those with any record of prescription for citalopram during their follow-up time (ever citalopram). We used a similar procedure to classify cases and controls as ever or never users of another SSRI or of another prescription medication that is a *CYP2D6* inhibitor or substrate.

For ER+/TAM+ women who ever had a citalopram prescription, we calculated the percentage of time on tamoxifen during which they were simultaneously taking citalopram. We created categories of (a) intermittent citalopram use, defined as citalopram use overlapping tamoxifen use for more than 0% but less than 30% of the time on tamoxifen, and (b) regular citalopram use, defined as citalopram use overlapping tamoxifen use for 30% or more of the time on tamoxifen. For this analysis, we used the full duration of their tamoxifen use as recorded in the DGCG registry, which was often longer than the duration anticipated by their original protocol assignment.

Covariates. We defined the following set of covariates: time period of breast cancer diagnosis, age at diagnosis, menopausal status at diagnosis, county of residence at diagnosis, UICC stage at diagnosis, histologic grade, surgery type, receipt of systemic adjuvant chemotherapy, and receipt of a prescription for another medication that is a *CYP2D6* inhibitor or substrate while taking tamoxifen (aside from those used to treat breast cancer recurrence or its effects).

Analytic strategy

We performed all analyses within strata of ER+/TAM+ and ER−/TAM− women. We calculated the number of cases and controls ever receiving each SSRI, the number of total prescriptions for each SSRI summed over all cases or controls, and the range of the number of prescriptions for each SSRI received by each individual case or control. Table I gives a complete list of SSRI medications and the frequency of their use in the study population. We also classified cases and controls as ever or never users of another prescription medication that is a *CYP2D6* inhibitor or substrate. Table II gives a complete list of these medications and the frequency of their use in the study population.

We then computed the frequency and proportion of cases and controls within categories of assigned protocol of tamoxifen duration, citalopram use, use of other SSRIs, use of other *CYP2D6* inhibitors or substrates, and the covariates.

We estimated the rate ratio associating citalopram prescription with breast cancer recurrence as the

Table I. Patterns of prescriptions for each SSRI.

	ERP+/TAM+n[a], (# of prescriptions[b]), [range of # per person[c]]		ERP−/TAM− n[a], (# of prescriptions[b]), [range of # per person[c]]	
SSRI name (ATC Code)	cases	controls	cases	controls
Zimeldine (N06AB02)	0	0	0	0
Fluoxetine (N06AB03)	5 (24) [2–11]	7 (60) [1–32]	2 (12) [1–11]	4 (19) [1–9]
Citalopram (N06AB04)[d]	33 (400) [1–53]	33 (163) [1–24]	12 (119) [1–35]	14 (120) [1–43]
Paroxetine (N06AB05)	6 (23) [1–13]	4 (16) [1–11]	1 (2) [2–2]	5 (39) [5–14]
Sertraline (N06AB06)	13 (86) [1–24]	15 (85) [1–18]	6 (28) [1–11]	4 (78) [1–48]
Alaproclate (N06AB07)	0	0	0	0
Fluvoxamine (N06AB08)	0	0	0	0
Etoperidone (N06AB09)	0	0	0	0
Escitalopram (N06AB10)[d]	5 (15) [1–6]	4 (18) [1–12]	0	0

[a]Number of cases and controls receiving any prescription for each SSRI.
[b]Total number of prescriptions for each SSRI.
[c]Range of number of prescriptions per person within women expressing the estrogen receptor and receiving at least one year of tamoxifen therapy (ERP+/TAM+), or not expressing the estrogen receptor, never receiving tamoxifen therapy, and surviving at least one year after diagnosis (ERP−/TAM−).
[d]In the analysis, we defined citalopram exposure as any prescription for citalopram (N06AB04) or its s-stereoisomer escitalopram (N06AB10).

Table II. *CYP2D6* inhibitors, substrates, and inducers used to adjust the association between breast cancer recurrence and ever/never use of citalopram or other SSRI.

Drug action & ATC Name	ATC code	ERP+/TAM+ cases/controls	ERP−/TAM− cases/controls
Histamine blocker			
Cimetidine	A02BA01	9/9	3/4
Ranitidine	A02BA02	0/2	2/0
Antiemetic			
Metoclopramide	A03FA01	12/5	12/1
Ondansetron	A04AA01	2/0	0/0
Antifungal			
Terbinafine	D01BA02	0/2	0/1
Antiarrythmia			
Flecainid	C01BC04	1/0	0/0
Amiodarone	C01BD01	0/0	0/1
Beta blocker			
Propranolol	C07AA05	5/0	2/1
Metoprolol	C07AB02	10/0	3/4
Timolol	S01ED01	3/0	0/0
Antihypertensive			
Carvedilol	C07AG02	1/0	1/0
Non-steroidal antiinflammatory			
Celecoxib	M01AH01	12/7	4/1
Analgesic			
Tramadol[a]	N02AX02	45/18	0/0
Codeine[a]	R05DA04	12/91	0/0
Oxycodone[a]	N02AA05	2/0	0/0
Antipsychotic			
Chlorpromazin	N05AA01	0/0	0/2
Levomepromazin	N05AA02	1/1	3/1
Haloperidol	N05AD01	1/0	1/0
Zuclopenthixol	N05AF05	1/1	2/2
Perphenazine	N05AB03	0/1	2/0
Risperidone	N05AX08	0/1	0/0
TCA Antidepressants			
Clomipramine	N06AA04	0/0	0/1
Amitriptyline	N06AA09	3/7	5/3
Nortriptyline	N06AA10	0/2	4/1
Other Antidepressants			
Moclobemid	N06AG02	1/0	0/0
Mirtazapin	N06AX11	9/10	4/2
Venlafaxin	N06AX16	6/2	0/1
Opioids			
Methadone[a]	N07BC02	0/1	0/0
Cough Suppressants			
Dexthromethorphan	R05DA09	0/1	0/0
Steroid Hormone			
Dexamethasone[a]	S01BA01	2/1	0/0

[a]Not included in the adjustment for ever/never use of a CPY2D6 inhibitor or substrate because the drug may be used to treat breast cancer recurrence or its symptoms.

odds ratio (OR) and its accompanying 95% confidence interval (CI) in a conditional logistic regression including only citalopram use as the exposure variable and conditioned on the matched factors. We then adjusted for additional confounding by covariates not included in the matching by including them as independent variables in a conditional logistic regression, retaining any covariate that affected the log odds ratio by more than ten percent. All analyses were performed using SAS version 9.

Results

Table I shows the pattern of SSRI prescriptions received by cases and controls. In both ER+/TAM+ and ER−/TAM− women, SSRI prescriptions were primarily written for citalopram or escitalopram. Table II shows the frequency and proportion of cases and controls who received prescriptions for other CYP2D6 substrates and inhibitors, within strata of ER+/TAM+ and ER−/TAM−. These frequencies and proportions were approximately the same among cases and controls, varying only as expected due to chance [20].

Table III shows the frequency and proportion of cases and controls, within strata of ER+/TAM+ and ER−/TAM−, in the categories of the covariates. Ten percent of ER+/TAM+ cases and 10% of ER+/TAM+ controls ever used citalopram while taking tamoxifen and about 6% of ER+/TAM+ cases and 6% of their controls ever used another SSRI while taking tamoxifen.

ER+/TAM+ women who ever used citalopram while taking tamoxifen had about the same rate of breast cancer recurrence as women who never used citalopram while taking tamoxifen (Table IV; adjusted OR=1.1, 95% CI=0.7, 1.7). These near-null results persisted within categories of intermittent and regular users of citalopram while taking tamoxifen. ER+/TAM+ women who ever used another SSRI (fluoxetine, paroxetine, or sertraline) while taking tamoxifen were also at no increased risk of breast cancer recurrence (Table IV; adjusted OR=0.9, 95% CI=0.5, 1.8). Neither citalopram use (adjusted OR=0.9, 95% CI=0.4, 2.2) nor use of another SSRI (adjusted OR=0.6, 95% CI=0.3, 1.6) had a substantial effect on recurrence in ER−/TAM− women, suggesting that these SSRI medications do not directly affect the risk of breast cancer recurrence.

Discussion

The results of this study provide clinical epidemiologic support for the hypothesis that citalopram, taken concurrently with tamoxifen, does not reduce tamoxifen's protective effect against breast cancer recurrence in early stage patients whose tumor cells express the estrogen receptor. This support is in agreement with recent recommendations that tamoxifen-treated breast cancer patients with indications for antidepressant medications may be safely prescribed citalopram or another SSRI with low potency to inhibit *CYP2D6* activity [12], and fills a void in the evidence base identified by the US

Table III. Frequency and proportion of cases of breast cancer recurrence and matched controls.

	ERP+/TAM+ [n, (%)]		ERP−/TAM− [n, (%)]	
	cases	controls	cases	controls
Citalopram prescription				
Ever	37 (10)	35 (10)	12 (5.3)	14 (6.1)
Ever, 0 to <30%[a]	24 (6.6)	25 (6.8)	7 (3.1)	10 (4.4)
Ever, 30 to ≤60%	6 (1.6)	7 (1.9)	1 (0.4)	1 (0.4)
Ever, >60%	7 (1.7)	3 (0.8)	4 (1.8)	3 (1.3)
Never	329 (90)	331 (90)	216 (95)	214 (94)
Other SSRI (ever exposed)				
Fluoxetine	5 (1.4)	7 (1.9)	2 (0.9)	4 (1.8)
Paroxetine	6 (1.6)	4 (1.1)	1 (0.4)	5 (2.2)
Sertraline	13 (3.6)	15 (4.1)	6 (2.6)	4 (1.8)
Other SSRI or *CYP2D6* inhibitor[c]				
Ever	103 (28)	95 (26)	53 (23)	54 (24)
Never	263 (72)	271 (74)	175 (77)	174 (76)
Diagnosis year[b]				
1985–1993	33 (9.0)	34 (9.3)	13 (5.7)	11 (4.8)
1994–1996	96 (26)	96 (26)	78 (34.2)	75 (33)
1997–2001	237 (65)	236 (65)	137 (60)	142 (62)
Age at diagnosis				
35–44	18 (4.9)	18 (4.9)	41 (18)	33 (15)
45–54	93 (25)	85 (23)	100 (44)	85 (37)
55–64	191 (52)	178 (49)	61 (27)	75 (33)
65–70	64 (18)	85 (23)	26 (11)	35 (15)
Menopausal status at diagnosis[b]				
Premenopausal	42 (12)	42 (12)	83 (36)	83 (36)
Postmenopausal	324 (89)	324 (89)	145 (64)	145 (64)
County of residence at diagnosis[b]				
Funen	61 (17)	61 (17)	47 (21)	47 (21)
South Jutland	41 (11)	41 (11)	29 (13)	29 (13)
Ribe	7 (1.9)	7 (1.9)	9 (3.9)	9 (3.9)
Vejle	38 (10)	38 (10)	43 (19)	43 (19)
Ringkøbing	13 (3.6)	13 (3.6)	4 (1.8)	4 (1.8)
Aarhus	83 (23)	83 (23)	42 (18)	42 (18)
Viborg	33 (9.0)	33 (9.0)	17 (7.5)	17 (7.5)
North Jutland	90 (25)	90 (25)	37 (16)	37 (16)
UICC tumor stage at diagnosis[b]				
Stage I	14 (3.8)	14 (3.8)	34 (15)	34 (15)
Stage II	148 (40)	148 (40)	111 (49)	111 (49)
Stage III	204 (56)	204 (56)	83 (36)	83 (36)
Histologic grade				
Grade I	59 (16)	89 (24)	19 (8.3)	13 (5.7)
Grade II	157 (43)	158 (43)	83 (36)	67 (29)
Grade III	78 (21)	45 (12)	90 (40)	90 (40)
Missing	72 (20)	74 (20)	36 (16)	58 (25)
Surgery type				
Breast conserving surgery	53 (15)	63 (17)	42 (18)	46 (20)
Mastectomy	313 (86)	303 (83)	186 (82)	182 (80)
Radiation therapy				
Yes	159 (43)	161 (44)	108 (47)	104 (46)
No	207 (57)	205 (56)	115 (50)	106 (47)
Missing			5 (2.2)	18 (7.9)
Tamoxifen protocol				
One year	76 (21)	59 (16)	Not Applicable	Not Applicable
Two years	50 (14)	62 (17)		
Five years	240 (66)	245 (67)		
Systemic adjuvant chemotherapy				
Yes	34 (9.3)	39 (11)	175 (77)	150 (66)
No	332 (91)	327 (89)	53 (23)	78 (34)

[a]Percent overlap of SSRI and tamoxifen prescription.
[b]Variable included in risk set sampling to match controls to cases.
[c]See Tables I and II for complete lists of SSRI and other *CYP2D6* inhibitors.

Table IV. Association between SSRI prescription and breast cancer recurrence.

Prescription	cases/controls	crude OR (95% CI)	adjusted OR (95% CI)[a]
ERP+/TAM+			
Never citalopram user	329/331	1 (reference)	1 (reference)
Ever citalopram user	37/35	1.1 (0.7, 1.7)	1.1 (0.7, 1.7)
Intermittent use	24/25	1.0 (0.5, 1.7)	1.0 (0.5, 1.7)
Regular use	13/10	1.3 (0.6, 3.0)	1.3 (0.6, 3.1)
ERP−/TAM−			
Never citalopram user	216/214	1 (reference)	1 (reference)
Ever citalopram user	12/14	0.8 (0.4, 1.9)	0.9 (0.4, 2.2)
ERP+/TAM+			
Never other SSRI user	345/344	1	1
Ever other SSRI[b] user	21/22	1.0 (0.5, 1.8)	0.9 (0.5, 1.8)
ERP−/TAM−			
Never other SSRI user	219/215	1	1
Ever other SSRI[b] user	9/13	0.7 (0.3, 1.6)	0.6 (0.3, 1.6)

[a]Adjusted for age category and other *CYP2D6* inhibiting medications (see Tables I and II for complete lists of these medications and the frequency of their use in the study population).
[b]Other SSRI are fluoxetine, paroxetine, and sertraline. See Table I for a description of their prescription frequencies.

National Comprehensive Cancer Network's treatment guidelines [21].

Most SSRI prescriptions in our study were for citalopram or its s-stereoisomer, which is a modest inhibitor of *CYP2D6* compared with some other SSRI medications [11,18,19]. Use of other SSRI medications (fluoxetine, paroxetine, or sertraline) while taking tamoxifen, some of which are more potent inhibitors of *CYP2D6* [11,22], was also unassociated with recurrence risk in our results. The frequencies of prescriptions for these other SSRIs were, however, too low to say with confidence that they do not reduce the effectiveness of tamoxifen.

This study extends our earlier results [13] by including 366 ER+/TAM+ cases and their 366 matched controls, resulting in 37 cases and 35 controls who ever used citalopram while taking tamoxifen. The earlier study included only 184 ER+/TAM+ cases and their 184 matched controls, resulting in only 17 cases and 21 controls who ever used citalopram while taking tamoxifen. Fifty-six percent of ER+/TAM+ cases and controls in this study were included in the earlier study, and 46% of citalopram-exposed cases were included in the earlier study. The present study's null result is, therefore, much more precisely measured than the null result of the earlier study. In addition, the large sample size and comprehensive prescription registry allowed investigation of, and control for, exposure to a wide range of prescription medications.

Despite the study's size and methodologic strength as a population-based case-control study, the results should be interpreted with the following limitations in mind. First, we do not know the reasons why SSRIs were prescribed to the study participants. SSRIs may have been prescribed to treat either depression or hot flashes [23], but SSRI prescriptions for hot flashes are very rare in Danish breast cancer patients. Second, we do not know whether participants carried *CYP2D6* variant alleles that reduce the enzyme's activity. Genetic variation in *CYP2D6* function, however, is not related to switching SSRI antidepressants or discontinuation of SSRI antidepressants [24], and does not affect response to, or tolerance of, citalopram in particular [25]. If *CYP2D6* genotype is unrelated to receipt or adherence to citalopram prescription, then the absence of genotyping data could not bias the results. Furthermore, clinicians caring for breast cancer patients who present with indications for SSRI antidepressants will seldom know the patient's *CYP2D6* genotype, so this study's result applies directly to the typical clinical setting.

Third, we have not confirmed that patients actually took either tamoxifen or a prescribed SSRI. In Denmark, tamoxifen is dispensed by breast cancer physicians to breast cancer patients at follow-up visits. SSRI medications recorded in the prescription registry are paid for and retrieved by patients, and then partly reimbursed by the national health care system. Both of these systems should assure good adherence to the registered medications. Fourth, most women taking SSRI prescription medications did not take them for the full duration of their tamoxifen therapy. This pattern reflects the clinical practice in this population during the study period. It would be very difficult to find a population in which a substantial proportion of tamoxifen-treated breast cancer patients took SSRI medications for the full five years of their tamoxifen therapy. Indeed, no such study has been reported. Finally, breast cancer patients with estrogen-receptor positive tumors were assigned treatment protocols calling for one, two, or five years of tamoxifen therapy, whereas cur-

rent guidelines recommend five years of tamoxifen therapy [21]. Many of the women assigned to tamoxifen protocols shorter than five years took tamoxifen for much longer (unpublished validation data), and we recorded the full duration of their use in the analysis of intermittent and regular use. In addition, recurrence risks between tamoxifen-treated and placebo-treated women differ as early as one year after initiation of tamoxifen treatment [8], so inhibition of tamoxifen effectiveness by concurrent SSRI prescriptions should have been apparent among all women included in our study.

While these results may seem at odds with the strong biologic rationale and *in vivo* evidence supporting the hypothesis that any *CYP2D6* inhibition would reduce tamoxifen's effectiveness, this information may not be as compelling as it first seems [26]. SSRI medications could reduce the plasma concentration of tamoxifen's secondary metabolites without reducing its anti-tumorigenicity [27]. Tamoxifen doses as low as 1 mg/day affect biomarkers of cardiovascular, bone, and tumor endpoints to about the same degree as the usual dose of 20 mg/day [28,29], so the three-fold reduction in the concentration of tamoxifen's secondary metabolites associated with receipt of the SSRI paroxetine [22] may have little consequence. Our results, combined with this emerging alternative view of the limited potential for *CYP2D6* inhibition to interact with tamoxifen, suggest that breast cancer patients with indications for an SSRI may be prescribed citalopram while taking tamoxifen with little effect, if any, on their risk of breast cancer recurrence.

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Null Results in Brief

No Increase in Breast Cancer Recurrence with Concurrent Use of Tamoxifen and Some *CYP2D6*-Inhibiting Medications

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Abstract

Tamoxifen reduces recurrence risk among women treated for estrogen receptor–positive breast cancer. Its effectiveness partly depends on metabolic activation via cytochrome P450 2D6 (*CYP2D6*). Some medications compromise *CYP2D6* activity and may lower plasma concentrations of active tamoxifen metabolites. We studied the association between concurrent use of tamoxifen and *CYP2D6*-inhibiting medications and breast cancer recurrence among Danish women diagnosed with early-stage, estrogen receptor–positive breast cancer. Using the Danish Breast Cancer Cooperative Group Registry, we identified 366 cases with local or distant breast cancer recurrence and 366 matched breast cancer controls. We ascertained concurrent prescription of *CYP2D6*-inhibiting medications during tamoxifen treatment by linking to the national prescription database covering all Danish pharmacies. We computed the breast cancer recurrence odds ratio (OR) and 95% confidence interval for each medication. The pooled recurrence OR was null (OR, 1.0; 95% confidence interval, 0.8-1.3); recurrence ORs for individual drugs ranged from 0.3 to 3.4. The individual ORs followed the pattern expected under a null-centered Gaussian distribution. Null associations were apparent for all drugs after empirical Bayes adjustment for multiple comparisons. Together, these results provide evidence for a null association between drug-compromised *CYP2D6* activity and breast cancer recurrence among tamoxifen-treated women. (Cancer Epidemiol Biomarkers Prev 2009;18(9):OF1–3)

Introduction

Tamoxifen approximately halves the 5-year recurrence risk among women treated for estrogen receptor–positive breast cancer (1). Cytochrome P450 enzymes metabolize tamoxifen to 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen, which exert the main pharmacologic effect (2-4). The gene encoding the cytochrome P450 enzyme chiefly responsible for 4-hydroxylation of tamoxifen, *CYP2D6*, is polymorphic and variant genotypes confer varying degrees of enzymatic impairment (5). Other medications inhibit, or are competing substrates for, *CYP2D6* activity (6, 7).[3] Tamoxifen-treated patients who also take potent *CYP2D6*-inhibiting drugs have low plasma concentrations of 4-hydroxy-*N*-desmethyltamoxifen, equivalent to concentrations in women with no functional *CYP2D6* allele (4, 8, 9). Current epidemiologic evidence is inconclusive regarding the effect of compromised *CYP2D6* function on the effectiveness of tamoxifen in preventing breast cancer recurrence (10). Here, we examine whether the use of *CYP2D6*-inhibiting medications was associated with higher breast cancer recurrence rates among tamoxifen-treated Danish women diagnosed with estrogen receptor–positive breast cancer.

Materials and Methods

This study was approved by the Boston University Medical Campus Institutional Review Board, the Regional Committee on Biomedical Research Ethics of Aarhus County, and by the Danish Registry Board.

Study Population. A description of study enrollment criteria and data collection procedures appear in an earlier publication (11). To summarize, we used the Danish Breast Cancer Cooperative Group Registry to identify women diagnosed with International Union Against Cancer stage I, II, or III breast cancer between 1994 and 2001 (12). Women were followed from 1 y after their diagnosis date until breast cancer recurrence, death from any cause, loss to follow-up, or September 1, 2006, whichever occurred first. We used the Danish Breast Cancer Cooperative Group Registry to identify cases of local or distant breast cancer recurrence among women with estrogen receptor–positive tumors who were treated with tamoxifen (n = 366). We selected one breast cancer control from each

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[3] http://www.medicine.iupui.edu/clinpharm/ddis/table.asp

Table 1. Observed associations between concurrent use of tamoxifen and *CYP2D6*-inhibiting medications and recurrence among Danish women diagnosed with estrogen receptor–positive breast cancer

	Exposed cases/ controls	Recurrence, OR* (95% CI)
CYP2D6-inhibiting medications (ever vs. never exposed)		
Celecoxib	8/15	0.3 (0.1-1.0)
Levomepromazine	2/4	0.5 (0.1-2.8)
Fluoxetine	5/7	0.6 (0.2-2.2)
Sertraline	13/15	0.7 (0.3-1.7)
Mirtazapine	14/16	0.8 (0.3-1.8)
Amitriptyline	6/8	0.8 (0.3-2.5)
Citalopram	33/33	0.9 (0.5-1.6)
Escitalopram	5/4	1.1 (0.3-4.7)
Metoclopramide	31/22	1.3 (0.7-2.4)
Cimetidine	16/14	1.4 (0.6-3.2)
Timolol	5/3	1.6 (0.4-6.9)
Propranolol	8/4	2.1 (0.5-8.7)
Venlafaxine	11/5	2.3 (0.7-7.2)
Paroxetine	6/4	2.4 (0.6-9.5)
Zuclopenthixol	5/2	3.4 (0.6-23)
	Total cases/ controls	Recurrence, OR† (95% CI)
Age at diagnosis (y)		
35-44	18/18	1.0 (Reference)
45-54	93/85	1.0 (0.5-2.2)
55-64	191/178	1.0 (0.4-2.3)
65-70	64/85	0.7 (0.3-1.7)
Tamoxifen protocol		
1 y	76/59	1.0 (Reference)
2 y	50/62	0.4 (0.2-0.9)
5 y	240/245	0.4 (0.1-1.2)

*Conditioned on matching factors and adjusted mutually for listed medications.
†Conditioned on matching factors.

recurrent case's risk set (13), matched on estrogen receptor expression, tamoxifen treatment status, county of residence, year of breast cancer surgery, menopausal status at diagnosis, and International Union Against Cancer stage at diagnosis. We defined the index date for each matched pair as the date of the case's breast cancer recurrence. Women received tamoxifen treatment for durations of 1, 2, or 5 y, depending on the prevailing Danish treatment protocol at the time of diagnosis (14).

Prescription Data Collection. We used the unique civil registration numbers of our breast cancer cases and controls to link the study roster to the national prescription database, which records drugs dispensed at all Danish pharmacies according to the Anatomical Therapeutic Chemical system.[4] We used Anatomical Therapeutic Chemical codes to ascertain prescriptions for medications known to be substrates for, or inhibitors of, *CYP2D6* activity[5] (a full list of searched drugs and Anatomical Therapeutic Chemical codes is available from the corresponding author). For each drug evaluated, cases and controls were classified as "ever exposed" to the drug if it was prescribed during their tamoxifen treatment; otherwise, they were classified as "never exposed."

Statistical Analysis. We tabulated the frequency of cases and controls according to use of *CYP2D6*-inhibiting medications, age group at diagnosis, and duration of tamoxifen use. We estimated breast cancer recurrence odds ratios (OR) and 95% confidence intervals (95% CI) associated with use of each of the concurrently prescribed medications using conditional logistic regression models, which addressed the matched factors and adjusted for confounders that changed the log OR estimates by >10% (15). We ranked the observed associations by magnitude and plotted the ORs against the inverse normal of rank percentile (16). On this plot, we overlaid predicted ORs from the inverse variance-weighted regression of observed log-odds values on the inverse normal of rank percentile. Finally, we subjected the vector of observed ORs to empirical Bayes adjustment for multiple comparisons (17, 18). Empirical Bayes adjustment shrinks individual associations toward the mean of a larger population of associations, in proportion to the ratios of the individual variances to the population variance. The method thus de-emphasizes imprecisely measured associations of otherwise striking magnitude, helping to avoid unproductive follow-up on what are likely to be false-positive findings.

Results

Of the candidate *CYP2D6*-inhibiting drugs we considered, 15 were prescribed to study subjects while they were taking tamoxifen. There were 120 cases and 103 controls who were exposed to at least one of the *CYP2D6*-inhibiting drugs while taking tamoxifen. Table 1 lists the conditional recurrence ORs for the 15 drugs. Recurrence ORs ranged from 0.3 (for celecoxib; 95% CI, 0.1-1.0) to 3.4 (for zuclopenthixol; 95% CI, 0.6-23). The recurrence OR pooled across all drugs was 1.0 (95% CI, 0.8-1.3). Figure 1 shows the plot of ORs against the inverse normal of rank percentile. The ascending diagonal line depicts the pattern under this plotting scheme that one would expect to observe if the vector of associations were drawn from an underlying null-centered Gaussian distribution (16). The observed drug associations fell almost perfectly along this line. Following empirical Bayes adjustment, no individual drug association differed appreciably from the pooled recurrence OR.

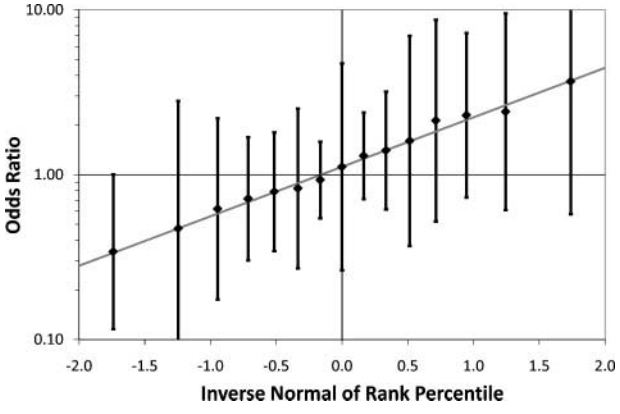

Figure 1. Distribution of ORs estimating the association between breast cancer recurrence and concurrent use of tamoxifen and *CYP2D6*-inhibiting medications, plotted against the inverse normal of each estimate's rank percentile. Medications are presented in the same order (*left to right*) as in Table 1.

[4] http://www.whocc.no/atcddd/
[5] http://www.medicine.iupui.edu/clinpharm/ddis/table.asp

Discussion

Our results do not support the hypothesis that the studied *CYP2D6*-inhibiting medications diminish the effectiveness of tamoxifen at reducing breast cancer recurrence among women treated for estrogen receptor–positive breast cancer. This study had 85% power to detect a statistically significant ($\alpha = 0.05$) 1.6-fold increase in the breast cancer recurrence rate among tamoxifen-treated women exposed to at least one of the drugs we examined. Furthermore, we had 99% power to detect a 1.9-fold increase in recurrence rate, which is the effect size observed in a recent report of concurrent use of tamoxifen and SSRI antidepressants (19). Because our study drew from the entire Danish breast cancer patient population during the study period, with complete follow-up, the study was not susceptible to selection bias. The prospectively collected Danish registry data reduced the risk of differential measurement error.

Nevertheless, the study has some limitations. First, we could not directly observe prescription compliance for both tamoxifen and *CYP2D6* inhibitors. Because prescriptions are only recorded in the registry after a medication has been paid for and dispensed, we expect prescription compliance to be high. An earlier validation study of hormone replacement therapy exposure classification by Danish prescription registries supports this expectation (20). Second, the duration of tamoxifen treatment differed within our study population according to prevailing treatment protocols during the study period (14). Because the protective effect of tamoxifen on recurrence manifests after the first year of treatment (1), we would expect ample opportunity for a modifier of its effectiveness to exert an effect, even among the small proportion of those in our study with the shortest assigned tamoxifen treatment regimen.

Disclosure of Potential Conflicts of Interest

The Department of Clinical Epidemiology at Aarhus University is involved in studies with funding from various companies as research grants to, and administered by, Aarhus University. These include a grant from the Lundbeck Foundation to study meningococcal disease and collaborations with the Centre for Registry Research, which receives grants from H. Lundbeck A/S (the manufacturer of citalopram and escitalopram). None of these studies have direct relation to the present study or supported any of the work reported herein.

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ORIGINAL PAPER

Lifetime tobacco smoke exposure and breast cancer incidence

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Abstract
Purpose We analyzed data from a case–control study to assess the association between lifetime tobacco smoke exposure and breast cancer incidence.
Methods Incident breast cancer cases were identified in the Massachusetts Cancer Registry and population controls were sampled from state Medicare lists and driver's license rosters. Demographic, lifestyle, medical history, reproductive history, and passive and active smoking exposure variables were assessed by telephone interview. We defined passive and active tobacco smoke exposure categories reflective of lifetime exposure patterns, and compared breast cancer risk among these groups while adjusting for age, body mass index, menopausal status, parity, alcohol consumption, and family history of breast cancer. We also adjusted passive smoking associations for active smoking status and vice versa.
Results We observed no association between ever being passively exposed to tobacco smoke and risk of incident breast cancer (adjusted OR: 1.2; 95% CI: 0.8, 1.8) nor between active smoking and breast cancer (adjusted OR for >23 pack-years compared to nonsmokers: 0.9; 95% CI: 0.7, 1.3). Null effects persisted in finer categorizations of active and passive exposure.
Conclusions We observed no causal associations between active or passive tobacco smoke exposures and incident breast cancer, consistent with results from most prospective cohort studies.

Keywords Breast neoplasms · Tobacco smoke pollution · Smoking · Epidemiology

Abbreviations

BMI	Body mass index
CI	Confidence interval
ETS	Environmental tobacco smoke
MCR	Massachusetts Cancer Registry
OR	Odds ratio

Introduction

Active and passive tobacco smoke exposures are hypothesized to be modifiable risk factors for female breast cancer, a disease with few known mutable causes [1]. Several studies have assessed the tobacco smoke and breast cancer association in various populations and with varying thoroughness of exposure assessment. Early studies showing little or no association between breast cancer risk and overall tobacco smoke exposure were criticized for inclusion of passively exposed persons in the reference group of never-active smokers, which perhaps attenuated an underlying positive association with exposure to tobacco smoke from any source [2]. Some early studies of passive tobacco smoke exposure were also criticized for failing to capture important sources of lifetime exposure (e.g., accounting for

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exposure at home, but not at the workplace), which might also have masked a truly elevated breast cancer risk [1]. A majority of studies have shown null associations between active tobacco smoke exposure and breast cancer [3–9]. However, results from studies of the association between passive tobacco smoke exposure and breast cancer have been conflicting, with several case–control studies reporting positive associations [2, 4, 5, 8–20].

Here, we present results from a population-based case–control study of the association between lifetime tobacco smoke exposure and incident female breast cancer among residents of Massachusetts. Our study is similar in design to two separate case–control studies of this association conducted in the same state [4, 13], but it more thoroughly characterizes lifetime tobacco smoke exposures.

Materials and methods

Study population

We conducted this investigation among the Massachusetts participants of a four-state breast cancer incidence study. The parent study assessed active tobacco smoke exposure by questionnaire; because of increasing interest in the potential role of passive tobacco smoke exposure in breast cancer development, new questions about these exposures were added while the study was underway. These new items were asked only of Massachusetts subjects who had yet to be interviewed by the time the questions were incorporated. The recruitment and selection criteria for the parent study are described in detail elsewhere [3, 21]. Briefly, women less than 75 years of age with incident invasive breast cancer were recruited from tumor registries in Massachusetts, New Hampshire, Maine, and Wisconsin between April 1989 and December 1991. For the current study, we ascertained incident breast cancer cases using the Massachusetts Cancer Registry (MCR), restricting eligibility to women who had a listed telephone number, a current driver's license, and a known date of diagnosis. Contact permission was sought from each eligible case's physician. Of 2,390 eligible cases, physicians refused contact permission for 234 (9.8%), 7 (0.3%) were not successfully located, 172 (7.2%) refused to participate, and 131 (5.5%) had died. This left 1,846 interviewed cases in the parent study, of whom 557 (30%) underwent the passive smoking assessment.

Controls were selected at random by two mechanisms: (1) controls under 65 years of age were sampled from annual Massachusetts driver's license rosters; (2) controls aged 65–74 were sampled from annual Massachusetts Medicare rosters. A total of 3,837 eligible controls were identified. Of these, 35 (0.9%) could not be located, 706 (18%) refused to participate, and 46 (1.2%) had died, yielding 3,050 interviewed controls in the parent study, of whom 432 (14%) underwent the passive smoking assessment.

Data collection

In addition to MCR data on cancer site, disease stage, and histologic features all participants were interviewed by telephone to assess demographic characteristics, reproductive history, hormone use, lifestyle factors, occupation, medical history, and lifetime passive and active exposures to tobacco smoke. Interviews were conducted by trained personnel who remained blind to the case–control status of 78% of cases and 90% of controls [3].

Definitions of analytic variables

Subjects were asked whether their parents smoked while they were living with them, and which parent smoked (mother, father, neither, or both). Subjects were also asked whether they had been exposed to tobacco smoke from other people for most of their adult life, including exposures at home and work, and the exposure intensity at each locale (characterized as never, occasionally, or regularly exposed). Overall exposure as an adult was considered positive if a subject reported occasional or regular exposure in at least one location. These interview responses served as discrete exposure definitions and were also assembled into concise lifetime passive exposure categories as never passively exposed, passively exposed as a child only, passively exposed as an adult only, or passively exposed as a child and as an adult.

An active smoking history was defined as having smoked $\geq$100 cigarettes over one's lifetime [e.g., 22]. Active smokers were asked to report their age at smoking onset, the daily average number of cigarettes smoked, and their age at smoking cessation (if they had quit before the interview). We calculated pack-years of cigarette smoking for each subject by multiplying years of active smoking by the usual number of cigarettes smoked per day, divided by 20 cigarettes per pack. Pack-years was categorized into approximate tertiles for stratified analyses.

All subjects were assigned an index date. For cases, this date was the date of breast cancer diagnosis. We calculated index dates for controls by subtracting the average interval between case diagnosis and interview from the interview date of each control. Age was calculated on the index date.

Parity was defined as the sum of live and still births at the time of interview. Body mass index (BMI) was calculated by dividing each woman's reported weight (kg) on the index date by her reported maximum lifetime height in meters, squared. Family history of breast cancer was considered positive if a woman reported a breast cancer

diagnosis in any first-degree female relative. Alcohol intake was characterized as the average grams of ethanol consumed per day between age 30 and 39 We calculated this by multiplying values of 12.8, 10.9, and 15.0 g of ethanol per serving by the reported daily consumption frequency of beer, wine, and liquor, respectively [23], and summing over beverage types.

We classified a woman as postmenopausal if she either (a) reported an age at menopause or (b) did not report an age at menopause, but was ≥50 years old on her index date, consistent with the mean age at natural menopause observed among a similar population of women [24]. All other subjects were classified as premenopausal women.

Statistical analysis

We calculated summary statistics for cases and controls according to demographic and clinical factors and tabulated the frequency of cases and controls according to passive and active tobacco smoke exposure. We modeled the association between passive and active tobacco smoke exposure and incident breast cancer using multivariate logistic regression. Covariates were evaluated as potential confounders based on a priori consideration of their association with both tobacco smoke exposure and breast cancer [25]. Namely, we considered age, BMI, menopausal status, parity, alcohol use, and family history of breast cancer. We also examined effect modification by menopausal status in a stratified multivariate analysis.

To address the potential impact of nondifferential misclassification on the association between ever/never passive tobacco smoke exposure and breast cancer, we simulated the impact of a range of sensitivity and specificity parameters for exposure classification on the observed result [26]. We anticipated self-classification of ever/never passive tobacco smoke exposure to have yielded many more false negatives than false positives, given the ubiquity of environmental tobacco smoke (ETS) during the childhood and adult lives of the subjects. We therefore hypothesized that individual classification specificities would range from 0.9 to 1.0, and individual sensitivities would range from 0.5 to 0.8. We assigned a uniform probability distribution to values in these ranges and performed 1,000 iterations of subject-level passive exposure misclassification adjustment, according to the methods of Fox et al. [26].

Odds ratios (OR) and Wald 95% confidence intervals (CI) were calculated for the comparisons listed in the tables. Multivariate logistic regression models were adjusted for age, BMI, menopausal status, parity, alcohol use, and family history of breast cancer. Associations between passive tobacco smoke exposure and breast cancer were additionally adjusted for active smoking and vice versa. Interaction between passive and active tobacco smoke exposure was assessed by inclusion of a cross-product term for these variables in a separate multivariate logistic regression model. All statistical tests were two-sided with $\alpha = 0.05$. All analyses were performed with SAS, version 9.1 (Cary, NC).

Results

Characteristics of cases and controls

Table 1 shows the distribution of key characteristics in cases and controls. Cases were slightly older, more often reported a family history of breast cancer, and consumed more alcohol than controls. Cases had lower average parity than controls, but were similar with regard to age at first birth among parous women. Among the entire study population, 445 (45%) subjects reported an age of menopause onset (mean reported age = 59.5 years; standard deviation = 6.2). There were 195 subjects with no reported age of onset who were classified as postmenopausal women by our age ≥50 criterion (mean age on index date = 55.6; standard deviation = 5.6).

Passive tobacco smoke exposure

Table 2 shows results for all categorizations of passive exposure. There was no association between ever having been regularly passively exposed to tobacco smoke and risk of breast cancer; the null effect persisted following adjustment for age, menopausal status, BMI, active smoking, parity, alcohol use, and family history of breast cancer (adjusted OR = 1.2, 95% CI: 0.8, 1.8). Results were similar, though less precisely estimated, when active smokers were removed from the reference and exposure groups. Of the candidate confounders, only age appreciably altered the log odds estimates upon adjustment; however, all changes were less than 10%, relative to unadjusted estimates.

For passive exposures due to parental smoking, we observed null effects when only the father smoked and when both parents smoked, but a positive association when only the mother smoked (adjusted OR = 1.9, 95% CI: 1.1, 3.3). Odds ratios were null for overall mother's smoking (i.e., irrespective of father's smoking status) and likewise for overall father's smoking (data not shown). Neither years lived with an active smoker as an adult nor places of passive exposure as an adult were associated with risk. When passive tobacco smoke exposures were assembled into categories reflecting lifetime exposure, effects were again null in all categories—even for women who were exposed as children, who may be most susceptible to the potential breast carcinogenic effect of tobacco smoke according to one model of susceptibility [13]. Our

Table 1 Characteristics of the study sample

Variable	Cases ($n = 557$)	Controls ($n = 432$)	χ^2 test for independence
Age (years) on reference date[a], *n* (%)			
28–35	13 (2.3)	17 (3.9)	$p = 0.02$
36–45	102 (18)	100 (23)	
46–55	202 (36)	158 (37)	
56–65	184 (33)	105 (24)	
66–75	56 (10)	52 (12)	
Missing	0	0	
Menopausal status on reference date, *n* (%)			
Premenopausal	191 (34)	158 (37)	$p = 0.45$
Postmenopausal	366 (66)	274 (63)	
Body mass index[b], *n* (%)			
16.5–18.5 (underweight)	7 (1.3)	6 (1.4)	$p = 0.98$
18.6–24.9 (normal)	273 (49)	213 (49)	
25.0–29.9 (overweight)	179 (32)	133 (31)	
≥30.0 (obese)	94 (17)	76 (18)	
Missing	4 (1)	4 (1)	
Family history of breast cancer[c], *n* (%)			
Yes	110 (20)	60 (14)	$p = 0.002$
No	427 (77)	367 (85)	
Missing	20 (3.6)	5 (1.2)	
Alcohol consumption (g/day in 30s)			
0	176 (32)	176 (41)	$p = 0.002$
1–12	314 (56)	220 (51)	
13–24	34 (6.1)	18 (4.2)	
≥25	25 (4.5)	7 (1.6)	
Missing	8 (1.4)	11 (2.6)	
Parity			
0	97 (17)	64 (15)	$p = 0.06$
1–3	384 (69)	283 (66)	
4–6	69 (12)	74 (17)	
≥7	6 (1.1)	11 (2.6)	
Missing	1 (0.2)	0	
Age at first birth (among parous women)			
12–19	47 (10)	53 (14)	$p = 0.19$
20–29	336 (73)	260 (71)	
30–34	46 (10)	38 (10)	
≥35	30 (6.5)	16 (4.4)	
Missing	0	1 (0.3)	

[a] Reference date for cases is their date of diagnosis. For controls, reference date was calculated as their date of interview minus the average interval between case diagnosis and case interview

[b] Weight (kg) on the reference date divided by the square of tallest lifetime height (m)

[c] Breast cancer diagnosed in mother, sister(s), or daughter(s)

simulation analysis of the impact of passive tobacco smoke exposure misclassification remained indicative of a null effect (simulated OR: 0.8, 95% simulation interval: 0.5, 1.2).

Active tobacco smoke exposure

We found no association between pack-years of cigarette smoking and incident breast cancer (Table 3). There was no substantial difference in the odds ratios whether or not the reference group contained passively exposed subjects and whether or not the active smoking estimates were adjusted for passive exposure. We observed no statistical interaction between passive and active tobacco smoke exposure ($p = 0.65$).

Menopausal status as an effect modifier

We examined menopausal status as a potential modifier of the odds ratio associating passive tobacco smoke exposure

Table 2 Associations between passive tobacco smoke exposure and incident breast cancer

Exposure categories	Cases ($n = 557$)	Controls ($n = 432$)	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
Ever/never exposure				
Never passively exposed	74	68	1. (Ref.)	1. (Ref.)
Ever passively exposed	483	364	1.2 (0.9, 1.7)	1.2 (0.8, 1.8)
Parental exposure				
Neither parent smoked	150	126	1. (Ref.)	1. (Ref.)
Both parents smoked	161	136	1.0 (0.7, 1.4)	1.1 (0.8, 1.6)
Only mother smoked	53	24	1.9 (1.1, 3.2)	1.9 (1.1, 3.3)
Only father smoked	191	145	1.1 (0.8, 1.5)	1.1 (0.8, 1.6)
Years lived with active smoker as an adult				
Not exposed as an adult	197	161	1. (Ref.)	1. (Ref.)
0 to <1	72	64	0.9 (0.6, 1.4)	0.9 (0.6, 1.3)
1–19	125	87	1.2 (0.8, 1.7)	1.1 (0.8, 1.6)
20–49	161	119	1.1 (0.8, 1.5)	1.0 (0.8, 1.6)
Places of passive exposure as an adult				
Not exposed as an adult	197	161	1. (Ref.)	1. (Ref.)
Work and home	188	130	1.2 (0.9, 1.6)	1.0 (0.8, 1.4)
Home only	97	84	0.9 (0.7, 1.4)	0.9 (0.6, 1.3)
Work only	70	55	1.0 (0.7, 1.6)	1.0 (0.6, 1.5)
Lifetime passive smoking exposure				
Never passively exposed	74	68	1. (Ref.)	1. (Ref.)
Exposed as child and as adult	284	212	1.2 (0.8, 1.8)	1.2 (0.8, 1.8)
Exposed as adult only	76	59	1.2 (0.8, 1.9)	1.1 (0.7, 1.8)
Exposed as child only	123	93	1.2 (0.8, 1.9)	1.3 (0.9, 2.1)
Lifetime passive smoking exposure (all ever actively exposed subjects excluded)				
Never actively or passively exposed	46	37	1. (Ref.)	1. (Ref.)
Exposed as child and as adult	92	72	1.0 (0.6, 1.7)	1.0 (0.5, 1.7)[b]
Exposed as adult only	31	27	0.9 (0.5, 1.8)	0.7 (0.4, 1.5)[b]
Exposed as child only	63	59	0.9 (0.5, 1.5)	1.0 (0.5, 1.7)[b]

[a] Adjusted for reference age (continuous), menopausal status (dichotomous), pack-years of active cigarette smoking (continuous), BMI at interview (continuous), parity (ordinal), average grams of alcohol consumed per day from age 30 to 39 (continuous), and family history of breast cancer (dichotomous)

[b] Not adjusted for pack-years of active cigarette smoking

with risk of breast cancer (Table 4). The odds ratio point estimates were slightly higher for all categories of passive exposure among postmenopausal women. We observed a modestly increased risk of breast cancer among postmenopausal women who were exposed only as children (OR: 1.8; 95% CI: 1.0, 3.3).

Discussion

In this population-based case–control study, we observed no material association between active and passive tobacco smoke exposure and risk of female breast cancer. Two exposure categories showed positive associations: women exposed to ETS as children through maternal smoking, and postmenopausal women exposed to ETS only as children. Additional data contradicted these associations: we further observed a null effect of childhood ETS exposure by both parents smoking, and there was a null effect of ETS exposure as both a child and an adult. These incongruities argue against causal relations for these exposure categories, since a true effect of passive exposure would not be expected to weaken when both of a subject's parents smoked or if there was exposure both as a child and as an adult.

The majority of past studies of active smoking and breast cancer risk report null associations [3–9], including a combined analysis of 53 studies with over 58,000 patients with breast cancer [27]. Studies of passive smoking and breast cancer risk are more evenly split between positive [2, 9, 11–13, 16, 18, 20] and null [4, 5, 8, 10, 14, 17, 19] associations. In a recent review, Johnson [1] identified five

Table 3 Association between active tobacco smoke exposure and incident breast cancer

Exposure categories	Cases ($n = 557$)	Controls ($n = 432$)	Crude OR (95% CI)	Adjusted[a] OR (95% CI)
Active exposures with reference group of never actively exposed subjects				
Pack-years > 23	136	106	1.1 (0.8, 1.5)	0.9 (0.7, 1.3)
(0 < pack-years ≤ 23)	189	131	1.2 (0.9, 1.6)	1.2 (0.9, 1.7)
Pack-years = 0	232	195	1. (Ref.)	1. (Ref.)
Active exposures with reference group of never passively or actively exposed subjects				
Pack-years > 23	136	106	1.0 (0.6, 1.7)	0.6 (0.3, 1.2)
(0 < pack-years ≤ 23)	189	131	1.2 (0.7, 1.9)	0.7 (0.4, 1.5)
Never actively or passively exposed	46	37	1. (Ref.)	1. (Ref.)

[a] Adjusted for reference age (continuous), menopausal status (dichotomous), lifetime regular passive tobacco smoke exposure (never, as child only, as adult only, as both child and adult), BMI (continuous), parity (ordinal), average grams of alcohol consumed per day from age 30 to 39 (continuous), and family history of breast cancer (dichotomous)

Table 4 Association between passive and active tobacco smoke exposure with incident breast cancer, stratified by menopausal status

Exposure categories	Premenopausal ($n = 349$)		Postmenopausal ($n = 640$)		$p_{interaction}$[d]
	Cases/controls	Odds ratio[c] (95% CI)	Cases/controls	Odds ratio[c] (95% CI)	
Lifetime passive smoking exposure[a]					
Exposed as child and adult	85/61	1.2 (0.7, 2.4)	199/151	1.3 (0.8, 2.2)	0.26
Exposed as adult only	16/16	0.7 (0.3, 1.8)	60/43	1.4 (0.7, 2.6)	
Exposed as child only	54/51	0.9 (0.5, 1.8)	69/42	1.8 (1.0, 3.3)	
Never passively exposed	36/30	1. (Ref.)	38/38	1. (Ref.)	
Pack-years of active smoking[b]					
Pack-years > 23	30/28	0.8 (0.4, 1.6)	106/78	1.0 (0.6, 1.4)	0.44
(0 < pack-years ≤ 23)	80/52	1.5 (0.9, 2.4)	109/79	1.1 (0.7, 1.6)	
Pack-years = 0	81/78	1. (Ref.)	151/117	1. (Ref.)	

[a] Odds ratios adjusted for pack-years of active cigarette smoking (continuous)

[b] Odds ratios adjusted for lifetime regular passive smoking exposure (never, as child only, as adult only, as both child and adult)

[c] All odds ratios adjusted for age (continuous), BMI (continuous), parity (ordinal), average grams of alcohol consumed per day from age 30 to 39 (continuous), and family history of breast cancer (dichotomous)

[d] Wald tests for the interaction terms between menopausal status and passive and active smoking exposures in the adjusted logistic regression model

epidemiologic studies deemed to have thorough passive smoking exposure assessment (inclusion of childhood home exposure, adult home exposure, and workplace exposure). The five studies, very similar in design to this study, each showed increased odds of breast cancer among women ever passively exposed to tobacco smoke, compared with women not passively or actively exposed (summary OR: 1.9; 95% CI: 1.5, 2.4), and increased odds of breast cancer among ever-active smokers, compared to never actively, never regularly passively exposed women (summary OR: 2.1; 95% CI: 1.4, 3.0) [1]. All five of the studies highlighted in Johnson's review assessed tobacco smoke exposure retrospectively—i.e., after the breast cancer cases had occurred—and were therefore susceptible to differential exposure misclassification. This bias would likely yield higher sensitivity of exposure classification among breast cancer cases, with an accompanying increase in false positives, thus biasing effect estimates upward [25].

A recent prospective cohort study of this association, in which tobacco smoke exposure was characterized thoroughly before breast cancer occurrence, showed null associations at all levels of active and passive tobacco smoke exposure [5]. A recent meta-analysis of prospective studies of the association between passive smoking and breast cancer (eight studies, but not including reference 5) found an overall null association (summary relative risk: 0.99; 95% CI: 0.93, 1.05) [28]. Our study, which characterized tobacco smoke exposures in the same manner as the five studies favored by Johnson [1] in his review, agrees with the findings of most prospective cohort studies as well as a large pooled analysis [27].

Limitations

Our null results must be considered with the limitations of our study in mind. We do not expect our administrative selection criteria (receipt of case contact permission from physicians, and the presence of controls on either Medicare or state driver's license rosters) to be strongly associated with tobacco smoke exposure, thus lessening the threat of selection bias via these exclusions. However, eligible cases and controls had to consent to participate, which may have been partly influenced by tobacco-related behaviors or comorbidities. This mechanism of selection bias is an implausible explanation for the observed null results. The relatively small proportion of cases and controls in the parent study who underwent the passive smoking assessment was a function of the late implementation of those interview questions during the study period, not of a self-selection mechanism.

Misclassification may have influenced our results. Tobacco smoke exposures were classified based on the self-reports of cases and controls. Since the study was of retrospective design (exposures were first recorded after the occurrence of disease), recall bias is a substantial threat to validity. If this recall bias scenario were operating in our study, the results would likely be an overestimate of the effect, because one would expect cases to report exposure with higher sensitivity than controls. The observed null associations and results of the sensitivity analysis argue against a large effect of this phenomenon.

Nondifferential exposure misclassification tends to attenuate truly non-null effects of dichotomous exposures. Our simulation analysis argues against a substantial influence of misclassification on the association between ever/never passive tobacco smoke exposure and breast cancer.

Residual confounding, either from failure to control for an important confounder or by misclassification of the covariates, is also unlikely to explain our results. None of the candidate confounders we evaluated resulted in a substantial change from the crude odds ratios upon adjustment. An important confounder that is both unmeasured and unassociated with the confounders we did measure, but for which adjustment would have had appreciable effect, seems unlikely to exist. Age had the largest effect upon adjustment and is not likely to have been substantially misclassified.

Conclusion

We found no associations between lifetime exposure to tobacco smoke and incident breast cancer. These results run counter to several similar retrospective studies that report a causal association between passive tobacco smoke exposure and breast cancer [2, 9, 11, 12, 20], but are consistent with studies that have shown similarly null effects, including most prospective cohort studies [5, 10, 19, 28] and a large combined analysis [27].

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METHODOLOGY

Potential misinterpretations caused by collapsing upper categories of comorbidity indices: An illustration from a cohort of older breast cancer survivors

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Background: Comorbidity indices summarize complex medical histories into concise ordinal scales, facilitating stratification and regression in epidemiologic analyses. Low subject prevalence in the highest strata of a comorbidity index often prompts combination of upper categories into a single stratum ('collapsing').

Objective: We use data from a breast cancer cohort to illustrate potential inferential errors resulting from collapsing a comorbidity index.

Methods: Starting from a full index (0, 1, 2, 3, and ≥4 comorbidities), we sequentially collapsed upper categories to yield three collapsed categorizations. The full and collapsed categorizations were applied to analyses of (1) the association between comorbidity and all-cause mortality, wherein comorbidity was the exposure; (2) the association between older age and all-cause mortality, wherein comorbidity was a candidate confounder or effect modifier.

Results: Collapsing the index attenuated the association between comorbidity and mortality (risk ratio, full versus dichotomized categorization: 4.6 vs 2.1), reduced the apparent magnitude of confounding by comorbidity of the age/mortality association (relative risk due to confounding, full versus dichotomized categorization: 1.14 vs 1.09), and obscured modification of the association between age and mortality on both the absolute and relative scales.

Conclusions: Collapsing categories of a comorbidity index can alter inferences concerning comorbidity as an exposure, confounder and effect modifier.

Keywords: epidemiology, breast neoplasms, comorbidity, confounding factors (epidemiologic), bias (epidemiologic), statistical models

Introduction

Proper accounting for comorbid diseases – medical conditions co-prevalent with a diagnosis of clinical or research interest[1] – has been a long-standing emphasis in the practice of clinical epidemiology. To this end, comorbidity indices have been developed to summarize complex medical histories in consolidated ordinal scales, offering statistical efficiency and straightforward interpretation compared with the inclusion of individual comorbid diseases in statistical models or stratified analyses.[2,3] The simplest comorbidity index is the sum of diseases co-prevalent with the studied diagnosis. This approach can be augmented by incorporating disease severity through empirical weighting systems.[2,4–6]

Regardless of the chosen index, comorbidity may be treated as an exposure, candidate confounder, or effect modifier in epidemiologic analyses. The prevalence

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of subjects in the highest categories of a comorbidity index is usually much lower than the prevalence in lower categories. The consequential data sparsity often motivates the combination of one or more upper categories into a single stratum. Lash recently explained the potentially hazardous consequences of collapsing upper categories of comorbidity indices for the sake of statistical efficiency or ease of interpretation.[7] Examples of such collapsing are common in the literature, even in studies with rather large sample sizes where data sparsity was not likely the chief inducement for doing so. For instance, Elkin and colleagues used a modified Charlson Comorbidity Index[2] to represent comorbidity as a confounder in an analysis of chemotherapy exposure and survival among older women with hormone receptor-negative breast cancer.[8] The Charlson Comorbidity Index is an ordinal variable ranging in value from 0 to 3, yet the authors combined the two highest categories (scores of 2 and 3) into one stratum with over 650 subjects. In a larger study of adjuvant chemotherapy in breast cancer, Giordano and colleagues also chose to collapse the two highest categories of the Charlson Comorbidity Index, yielding a collapsed stratum with over 3,800 subjects.[9] There are also published studies in which a collapsed comorbidity index was a primary epidemiologic exposure.[10–12]

Herein we illustrate the potentially hazardous consequences of collapsing upper categories of a comorbidity index[7] using data from the Breast Cancer Treatment in Older Women (BOW) cohort study.[13] We evaluate the association between a simple index of comorbidity and the risk of death from any cause, examine confounding by comorbidity of the association between older age and all-cause mortality, and assess modification of the age/mortality association by comorbidity. In all three scenarios we demonstrate the impact of collapsing upper categories of the comorbidity index on the inferences obtained under full categorization. We also illustrate a risk trend analysis using polynomial regression, a proposed alternative to categorical statistics for depicting dose-response relations between an exposure and an outcome.[14] Finally, we discuss restricting analyses to comorbidity categories of sufficient size as a simple alternative to collapsing.

Methods

Study population

We conducted this study in the BOW cohort of older women diagnosed with early stage breast cancer who were recruited from integrated health systems participating in the HMO Cancer Research Network (CRN).[15] The CRN consists of the research programs, enrollee populations, and databases of 14 members of the HMO Research Network. The main goal of the CRN is to conduct collaborative research to determine the effectiveness of preventive, curative, and supportive interventions for major cancers that span the natural history of those cancers among diverse populations and health systems.

Data collection procedures for the BOW cohort are thoroughly described in an earlier publication.[13] Briefly, women age ≥65 years with a histologically-confirmed first diagnosis of American Joint Commission on Cancer TNM stage[16] I or II breast cancer between January 1, 1990 and December 31, 1994 who were enrolled in six geographically diverse health systems (Group Health Cooperative, Seattle, Washington; Kaiser Permanente Southern California; Lovelace/Sandia Health System, New Mexico; Henry Ford Health System, Detroit, Michigan; HealthPartners, Minneapolis, Minnesota; and Fallon Community Health Plan, Worcester, Massachusetts) were identified either through population-based tumor registries or health system administrative data combined with medical record review. Women were excluded if they had been diagnosed with any other malignancy (other than nonmelanoma skin cancer) either five years before, or 30 days following, their breast cancer diagnosis. Women simultaneously diagnosed with contralateral breast cancer were also excluded. To address possible confounding by receipt of chemotherapy,[17] we restricted our analytic cohort to those women who did not receive chemotherapy.

The study protocol was approved by the institutional review boards at all participating organizations.

Data collection

Population cancer registries, clinical databases, and administrative databases were used in concert with medical record reviews to electronically collect demographic, tumor, treatment, and comorbidity data for enrolled subjects.[18] Comorbidities that were present in the year before breast cancer diagnosis were ascertained from medical records as part of a standard abstraction protocol. Date and cause of death were ascertained from the National Death Index.

Definition of analytic variables

For illustrative purposes, we constructed a simple index of comorbidity equal to the unweighted sum of health conditions prevalent in the year before breast cancer diagnosis. Diagnoses included in the index were heart failure, chronic pulmonary disease, connective tissue disease, cerebrovascular disease, dementia, diabetes, hemiplegia, hypertension, liver disease,

myocardial infarction, peripheral vascular disease, ulcer, and renal disease. These conditions, with the exception of hypertension, comprise a subset of the diagnoses encompassed by the Charlson Comorbidity Index.[2] Our subjects had between 0 and 7 comorbidities according to the simple index. The three highest categories were too sparsely populated to be considered independently (together they comprised ~1% of the persons at risk); we therefore defined our full index categorization as 0, 1, 2, 3, or ≥4 comorbidities. Beginning with this full index categorization, comorbidity categories were sequentially collapsed by adding counts from the highest and next-highest levels, until comorbidity was ultimately categorized dichotomously (≥1 or 0 comorbidities). This process yielded four categorizations of the comorbidity index; the full categorization plus three orders of collapsed categorization (eg, Table 1).

For regression modeling and describing baseline cohort characteristics, age was categorized as 65–69, 70–74, 75–79 and ≥80 years. For stratified analyses, age at breast cancer diagnosis was categorized dichotomously as ≥75 years old or 65–74 years old; this dichotomization provided a simple exposure categorization to use for our analyses of comorbidity as a confounder and modifier of the age/mortality association.

Tumor size was categorized as <1 cm, 1 to <2 cm, 2 to <3 cm, and ≥3 cm. Lymph node status was classified as positive or negative based on either histologic (n = 1311; 78%) or clinical evaluation (n = 276; 17%); 84 subjects (5.0%) were missing data on lymph node status. Adequate primary therapy was defined as having undergone either mastectomy or breast-conserving surgery with radiotherapy; other treatment regimens were classified as inadequate. Estrogen receptor (ER) status was classified as positive, negative, or indeterminate. Receipt of adjuvant tamoxifen therapy was classified as ever or never. To adjust for receipt of adjuvant tamoxifen, we created a composite variable by cross-tabulating ER status (positive/negative/indeterminate) with tamoxifen receipt (ever/never).

Mortality was defined as death from any cause occurring within the five years after breast cancer diagnosis.

Statistical analysis

We tabulated the frequency and risk of death from any cause and the total number of subjects according to age,

Table 1 Effects of serially collapsing upper categories of a comorbidity index on inferences regarding the association between comorbidity burden and five-year all-cause mortality risk

Comorbidity index categorization	Deaths	Total	Risk	$RR_{unadj.}$ (95% CI)[a]	$RR_{adj.}$ (95% CI)[a,b]
Full Index					
≥4	29	54	0.54	**4.6 (3.3, 6.4)**	**3.1 (2.1, 4.4)**
3	34	93	0.37	3.1 (2.2, 4.4)	2.6 (1.8, 3.7)
2	84	292	0.29	2.5 (1.8, 3.3)	2.1 (1.5, 2.8)
1	119	658	0.18	1.5 (1.2, 2.0)	1.3 (1.0, 1.8)
0	67	574	0.12	1	1
1st Order					
≥3	63	147	0.43	**3.7 (2.7, 4.9)**	**2.8 (2.0, 3.8)**
2	84	292	0.29	2.5 (1.8, 3.3)	2.1 (1.5, 2.8)
1	119	658	0.18	1.5 (1.2, 2.0)	1.3 (1.0, 1.8)
0	67	574	0.12	1	1
2nd Order					
≥2	147	439	0.33	**2.9 (2.2, 3.7)**	**2.3 (1.8, 3.0)**
1	119	658	0.18	1.5 (1.2, 2.0)	1.3 (1.0, 1.8)
0	67	574	0.12	1	1
3rd Order					
≥1	266	1097	0.24	**2.1 (1.6, 2.7)**	**1.7 (1.3, 2.2)**
0	67	574	0.12	1	1

Notes: [a]Risk ratios and 95% confidence limits were estimated by modified Poisson regression; [b]Adjusted for age category, tumor size, lymph node positivity, receipt of adequate primary therapy, and tamoxifen receipt according to estrogen receptor status. Eighty-four subjects were excluded from adjusted models due to missing node positivity data.

Abbreviations: CI, confidence intervals; RR, relative risk.

comorbidity count, and tumor and treatment characteristics at the time of breast cancer diagnosis (Table 2).

To assess comorbidity as an exposure variable, we tabulated frequencies and calculated risks of death from any cause within strata of all comorbidity categorizations (Table 1). Women with no comorbidity served as the reference group for all comparisons. We fit a modified Poisson regression model with robust standard error estimates to estimate mortality risk as a function of comorbidity level, with and without adjustment for age category, tumor size, lymph node status, adequacy of primary therapy, and adjuvant hormonal therapy (Table 1).[19] The 84 individuals with missing data for lymph node status were excluded from the multivariate models; results observed under this exclusion were nearly identical to those obtained after multiple imputation of the missing observations (data not shown).

To assess comorbidity as a candidate confounder or modifier, we conducted a stratified analysis of the association between age (≥75 vs 65–74 years) and all-cause mortality according to the comorbidity index under all categorizations (Table 3). Stratum-specific risk ratios (RR) and risk differences (RD) were calculated for the full and collapsed orders of the comorbidity index. For each comorbidity categorization, we calculated the standardized mortality risk ratio (SMR) across strata and divided this figure into the crude risk ratio (the unadjusted age/mortality association) to yield the relative risk due to confounding (RR_c), which measures the direction and magnitude of risk ratio distortion due to confounding by comorbidity. The popular 'change in estimate criterion' considers a change of >10% as indicative of substantial confounding by a candidate variable, indicating that it should be retained in either a stratified analysis or a multivariate regression model of the studied association.[20]

Modification of the age/mortality association by comorbidity index was assessed on both the difference and ratio scales. The interaction contrast (IC; modification of the risk difference) was calculated as the difference in risk-difference values between the highest and lowest comorbidity strata in each categorization. Effect measure modification (EMM; modification of the risk ratio) was calculated as the ratio of the risk-ratios in the highest and lowest comorbidity strata in each categorization.[21] A value of zero for the interaction contrast indicates no modification on the difference scale, while a value of one for effect measure modification indicates no modification on the ratio scale.[21]

Table 2 Baseline characteristics of cohort members (N = 1,671)

Characteristic	Number of deaths (risk)	Persons at risk, [n (%)]
Age at diagnosis (years)		
65–69	63 (0.12)	515 (31)
70–74	89 (0.18)	493 (30)
75–79	62 (0.21)	301 (18)
≥80	119 (0.33)	362 (22)
Number of comorbid conditions at breast cancer diagnosis		
7	1 (1.0)	1 (0.1)
6	4 (0.67)	6 (0.4)
5	7 (0.58)	12 (0.7)
4	17 (0.49)	35 (2.1)
3	34 (0.37)	93 (5.6)
2	84 (0.29)	292 (17)
1	119 (0.18)	658 (39)
0	67 (0.12)	574 (34)
Tumor characteristics		
Tumor size (cm)		
<1	45 (0.12)	371 (22)
1 to <2	118 (0.17)	712 (43)
2 to <3	95 (0.25)	375 (22)
≥3	75 (0.35)	213 (13)
Node status		
Positive	74 (0.22)	329 (20)
Negative	231 (0.18)	1,258 (75)
(Missing)	28 (0.33)	84 (5.0)
Treatment characteristics		
Primary therapy		
BCS+AND+RT or mastectomy	209 (0.16)	1,271 (76)
Other treatment	124 (0.31)	400 (24)
ER status/tamoxifen status		
ER+/tamoxifen–	70 (0.21)	338 (20)
ER+/tamoxifen+	166 (0.18)	916 (55)
ER-/tamoxifen–	19 (0.20)	95 (5.7)
ER-/tamoxifen+	31 (0.31)	100 (6.0)
ER indeterminate/ tamoxifen–	27 (0.21)	131 (7.8)
ER indeterminate/ tamoxifen+	20 (0.22)	91 (5.4)

Abbreviations: BCS+AND, breast conserving surgery with axillary node dissection; ER, estrogen receptor; RT, radiotherapy.

Table 3 Effects of serially collapsing upper categories of a comorbidity index on the assessment of confounding or effect measure modification by comorbidity of the association between age and five-year all-cause mortality

Comorbidity index categorization	Age: ≥75 deaths/total	Age: 65–74 deaths/total	Risk ratio	Risk difference	SMR[a]	RRc[b]	IC[c]	EMM[d]
Full index								
≥4	14/29	15/25	0.8	–0.12	1.59	1.14	–0.20	0.43
3	14/43	20/50	0.8	–0.07				
2	54/133	30/159	2.2	0.22				
1	69/285	50/373	1.8	0.11				
0	30/173	37/401	1.9	0.08				
1st Order								
≥3	28/72	35/75	0.8	–0.08	1.60	1.13	–0.16	0.44
2	54/133	30/159	2.2	0.22				
1	69/285	50/373	1.8	0.11				
0	30/173	37/401	1.9	0.08				
2nd Order								
≥2	82/205	65/234	1.4	0.12	1.63	1.11	0.04	0.77
1	69/285	50/373	1.8	0.11				
0	30/173	37/401	1.9	0.08				
3rd Order								
≥1	151/490	115/607	1.6	0.12	1.66	1.09	0.04	0.87
0	30/173	37/401	1.9	0.08				
Unstratified (crude)	181/663	152/1008	1.81	0.12	n/a	n/a	n/a	n/a

Notes: [a]Standardized mortality risk ratio; calculated as the ratio of observed to expected deaths, based upon the risk in those aged 65–74; [b]Relative risk due to confounding; calculated as the ratio of crude risk ratio and the categorization-specific SMR values; [c]Interaction contrast (modification of the risk difference); difference of the risk differences in highest and lowest comorbidity levels; [d]Effect measure modification (modification of the risk ratio); ratio of the risk ratios in highest and lowest comorbidity levels.

As an alternative to collapsing upper comorbidity categories to depict the association between comorbidity and all-cause mortality, we generated a cubic power function for mortality risk by maximizing the log-binomial likelihood of the observed data, using the entire range of comorbidity counts in the cohort (0 to 7 comorbidities).[14] We plotted the observed risks at each observed comorbidity count and overlaid the modeled function (Figure 1).

All analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

The BOW cohort enrolled 1,859 women. Exclusion of subjects who received chemotherapy yielded an analytic cohort of 1,671 women. The baseline characteristics of the analytic cohort are shown in Table 2. After five years, 333 subjects (20%) had died and 68 subjects (4.1%) disenrolled from their health care system. The prevalence of the two lowest categories of the comorbidity index, 0 and 1 comorbidity, were nearly equivalent (34% and 39%, respectively). Thereafter, comorbidity prevalence decreased with increasing index value; 17% of subjects had two comorbidities, 5.6% had three, and 3.2% had four or more.

Effect of collapsing comorbidity index on exposure inference

The five-year risk of death from any cause increased monotonically across levels of the full comorbidity index (Table 1), ranging from 12% for those with no comorbidities to 54% for those with four or more conditions. Compared with women with no comorbidity, those with four or more comorbidities had a 4.6-fold higher unadjusted risk of death over five years. As illustrated by the bolded risk ratios in Table 1, sequentially collapsing the highest comorbidity category into the next-highest category caused an attenuation of the measures of association between comorbidity and mortality, culminating in an unadjusted risk ratio of 2.1 (95% confidence interval [CI]: 1.6, 2.7) when comorbidity was dichotomized (the 3rd order categorization). The percent reduction in the estimated risk ratios, compared with the fully categorized comorbidity index, was 20%, 37%, and 54% for the first, second, and third collapsed

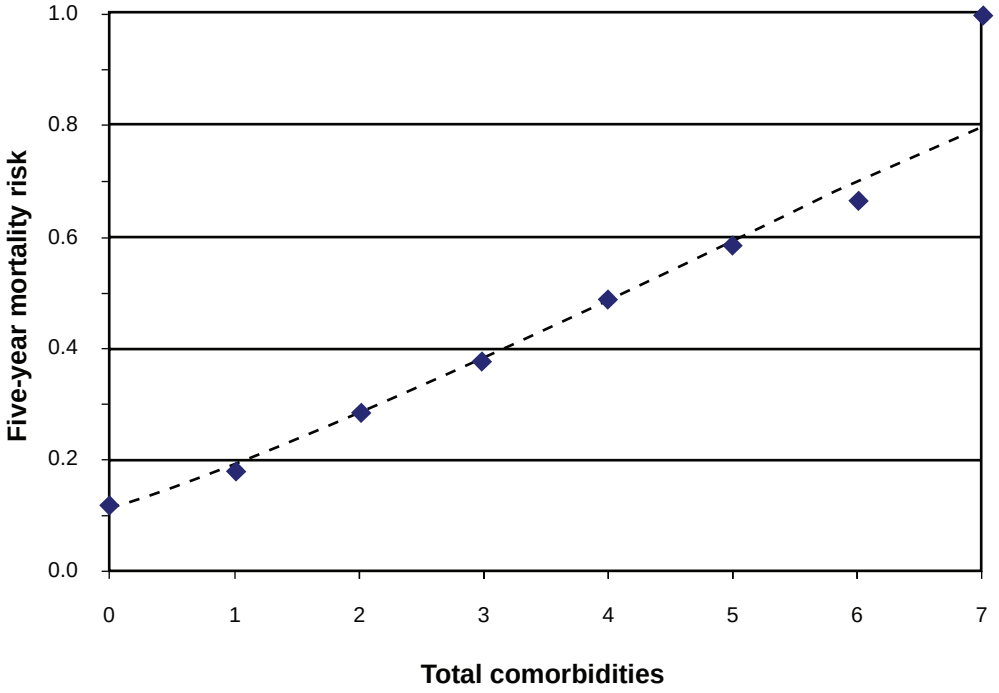

Figure 1 Plot of five-year mortality risk as a function of comorbidity count. The diamond markers denote observed risks for each comorbidity count. The dashed line depicts the risk trend described by a fitted cubic polynomial model.

orders, respectively. Thus, any degree of comorbidity index simplification substantially altered the magnitude of the association observed between comorbidity and the outcome.

Effect of collapsing comorbidity on the assessment of confounding

Table 3 shows associations between older age (≥75 vs 65–74 years) and five-year all-cause mortality within strata of different comorbidity index categorizations. The crude RR (unadjusted for comorbidity) for the association was 1.81. Standardized mortality risk ratios ranged from 1.59 for the full categorization to 1.66 for the dichotomized categorization; RR_c values ranged from 1.14 under the full categorization to 1.09 under the dichotomized categorization. Under full categorization, an investigator would conclude that there was substantial confounding by comorbidity, and would choose to retain it as an adjustment or stratification variable. This conclusion would also be reached under the first- and second-order collapsed categorizations. However, under the dichotomized categorization, an investigator might conclude that there was no substantial confounding by comorbidity ($0.9 < RR_c = 1.09 < 1.1$), and may elect to exclude comorbidity from stratified tables (to avoid sparsity) or from multivariate regression models (to improve parsimony).

Effect of collapsing comorbidity on the assessment of interaction

Table 3 also shows the calculated measures of interaction on both the absolute (RD) and relative (RR) scales. The interaction contrast (modification of the RD) equaled –0.20 under the fully categorized index, indicating that the highest index level and older age interacted to reduce mortality risk by 20 cases per 100 persons over the follow-up period, compared with the risk expected from the independent effects of age and comorbidity as well as the baseline risk. The interaction contrast approached the null upon sequential combination of upper comorbidity levels, ultimately equaling 0.04 under the dichotomized categorization. This value might lead an investigator to conclude that older age and comorbidity had interacted to increase mortality risk by four cases per 100 persons over the follow-up period – a measure five-fold lower in magnitude and of opposite sign to that obtained under full categorization.

Effect measure modification (modification of the RR) equaled 0.43 under full categorization, indicating that the RR associating age and mortality in the highest comorbidity stratum was 57% lower than the corresponding RR in the no-comorbidity stratum. Thus, an investigator would conclude that the association between older age and mortality varied in magnitude (and in direction as well, in this particular

example) according to level of comorbidity index. The EMM measure rose in value upon sequential combination of upper categories of comorbidity index, ultimately equaling 0.87 under the dichotomized categorization. This value might either lead to an under-appreciation of the degree of risk ratio modification by comorbidity status, or to an outright dismissal of such interaction, owing to the closeness of this value to unity.

A power model as an alternative to analyzing exposure effect

The dose-response plot in Figure 1 shows the mortality risk profile according to the full range of comorbidity counts observed in the cohort. The observed risks exhibited an approximately linear response pattern, affirmed by the fitted cubic polynomial function. This dose-response plot preserves the full range of exposure levels and their cognate responses, avoiding the pitfalls of collapsing exposure categories. Though our power model was univariate (comorbidity was the sole independent variable), such models can easily accommodate covariates of interest, yielding model-adjusted risk trends.[22]

Discussion

We used the sum of prevalent comorbidity diagnoses in the year before breast cancer diagnosis as our comorbidity index. While the simplicity of this index imparts limitations for its use as an analytic variable in an epidemiologic study, its role here is purely illustrative. Likewise, the association between older age and all-cause mortality was chosen for its demonstrative potential (eg, the strong associations between both age and death with comorbidity). We employed this trio of variables to demonstrate principles that may apply to other comorbidity scores, such as the widely used Charlson Comorbidity Index, and to other marginal associations of interest.

When comorbidity was treated as an exposure, we found that combining upper index categories attenuated measures of the association between comorbidity and all-cause mortality. Limiting combination to the two highest levels of the full index reduced the crude risk ratio by approximately one-fifth. Collapsing to the extreme case of dichotomization (any comorbidity vs none) reduced the risk ratio by more than half. The actual magnitude of reduction will vary depending on the specific index chosen, the prevalence of each index level, and the outcome risk for each level.[7] Such reductions place an investigator at risk of underestimating the association between comorbidity and a given outcome.

Combining index categories also affected the decision about whether to adjust for comorbidity when using the popular '10% change in estimate' approach for confounder selection. Using our fully categorized index, we saw that comorbidity confounded the association between older age and all-cause mortality, increasing the observed RR by 14% ($RR_c = 1.14$). Since this value is greater than the typical 10% cutoff for a relative change in effect estimate, comorbidity would be retained as a stratification variable or covariate in a statistical model. Our decision was different, however, when comorbidity was dichotomized. Under dichotomization, the apparent distortion due to confounding by comorbidity was 9%, implying that no adjustment for comorbidity is necessary. In our example, choosing not to adjust for comorbidity would yield a RR inflated 14% by uncontrolled confounding, compared with the RR adjusted for the full index. While this particular pattern is specific to our data, combining categories of a confounder will predictably dull the observed impact of the confounder on the studied association. That is, it will bias the relative risk due to confounding toward the null, compared with what would be observed under narrower categorization.[23]

Evaluation of comorbidity as a modifier of the association between older age and mortality showed a convergence of interaction measures – on both the relative and absolute scales – toward their null values. Under the fully categorized comorbidity index, modification was apparent for both the risk ratio and the risk difference, showing diminished associations when the highest comorbidity level interacted with older age. Both types of modification were mostly obscured under the second-order collapsing of comorbidity categories (0, 1, and $\geq$2 comorbidities), and almost completely obscured under the third order, dichotomization. The pattern we observed with effect modification should not be taken as illustrative of the expected bias pattern for all cases. Lash demonstrated that collapsing comorbidity generates an unpredictable and erratic pattern of effect modification – sometimes masking existing modification, and other times generating spurious modification.[7] In our data, the pattern happened to be orderly and convergent on null values.

In summary, the practice of collapsing sparse upper categories of a comorbidity index may have important effects on inferences concerning comorbidity as an exposure, candidate confounder, or effect modifier. These limitations should be kept in mind when working with comorbidity indices. One safeguard is to collapse only adjacent upper categories with equal or similar outcome risks.[7] When

feasible, alternative analyses such as power models or spline regression can also be adopted to preserve the rich details of studied associations. A simpler, though less desirable, solution is to restrict analyses to comorbidity categories with sufficient sample sizes. While this strategy limits analyses to persons with lower comorbidity scores, it avoids the potential for misinterpretation when high-comorbidity categories are collapsed into lower categories.

Disclosure

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Impact of Acquired Comorbidities on All-Cause Mortality Rates Among Older Breast Cancer Survivors

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Background: Breast cancer survivors with higher numbers of comorbidities at the time of primary treatment suffer higher rates of all-cause mortality than comparatively healthier survivors. The effect of time-varying comorbidity status on mortality in breast cancer survivors, however, has not been well investigated.

Objective: We examined longitudinal comorbidity in a cohort of women treated for primary breast cancer to determine whether accounting for comorbidities acquired after baseline assessment influenced the hazard ratio of all-cause mortality compared with an analysis using only baseline comorbidity.

Methods: Cox proportional hazards adjusted for age, race/ethnicity, and exercise habits were modeled using (1) only a baseline Charlson index; (2) 4 Charlson index values collected longitudinally and entered as time-varying covariates, with missing values addressed by carrying forward the prior observation; and (3) the 4 longitudinal Charlson scores entered as time-varying covariates, with missing values multiply imputed.

Results: The 3 modeling strategies yielded similar results; Model 1 HR: 1.4 per unit increase in Charlson index, 95% confidence interval (CI): 1.2–1.7; Model 2 HR: 1.3, 95% CI: 1.1–1.5; and Model 3 HR: 1.4, 95% CI: 1.2–1.6.

Conclusions: Our findings indicate that a unit increase in the Charlson comorbidity index raises the hazard rate for all-cause mortality by approximately 1.4-fold in older women treated for primary breast cancer. The conclusion is essentially the same whether accounting only for baseline comorbidity or accounting for acquired comorbidity over a median follow-up period of 85 months.

Key Words: aged, chronic disease, breast neoplasms, comorbidity, mortality

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Breast cancer is primarily a disease of older women, who frequently have other diseases as well.[1,2] When present, these diseases may affect breast cancer treatment choices and adherence to treatment regimens,[2–8] which would directly affect breast cancer mortality and therefore affect overall mortality. Medical attention focused on the treatment of breast cancer may also detract from definitive care of comorbid disease, and therefore increase all-cause mortality rates in breast cancer patients. Evidence for this phenomenon has been reported for serious diseases other than breast cancer.[9,10]

Recent years have witnessed a surge of investigations into the role comorbidity plays in the treatment and care of older cancer patients. Past studies have examined the effect of comorbid conditions on cause-specific and all-cause mortality rates, showing that older breast cancer survivors with a greater burden of comorbidity suffer from higher rates of all-cause mortality than those who are healthier.[4,6,8,11,12] To date, no study of this association has accounted for changes in comorbidity beyond the period of initial cancer treatment. We have expanded upon previous research by accounting for acquired comorbidity and examining its effect on all-cause mortality in a cohort of older women diagnosed with early stage breast cancer.

METHODS

Study Population

We conducted our study within an ongoing prospective cohort of older women diagnosed with early stage breast cancer. The enrollment criteria and data collection procedures for this cohort have been described in detail elsewhere.[13] Briefly, women aged 65 years or older diagnosed with early stage breast cancer (stage I with tumor diameter ≥1 cm, stage II, or stage IIIa) between 1996 and 1999 at 1 of 61 hospitals in Rhode Island, North Carolina, Minnesota, or Los Angeles, were identified through tumor registries and hospital pathology reports. Women whose physicians gave contact permission were invited to participate in the study (n = 1621**)**. Additional entry criteria included the following: (1) no prior history of primary breast cancer, (2) no simultaneously diagnosed primary tumor at another anatomic site, (3) English-speaking or with an available translator, and (4) competent for interview with satisfactory hearing or with an available proxy respondent. Women who were not enrolled within 5 months of the date of their breast cancer surgery were excluded. Of the 1621 women whose physicians gave contact permission, 865 consented to participate in the study and were subsequently enrolled. All participants returned a signed consent form approved by local institutional review boards.

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Participants were interviewed by telephone at 3, 6, and 15 months, and annually thereafter until 87 months, after primary tumor treatment. These interviews collected data on patient demographics, lifestyle, primary tumor and treatment characteristics, cancer recurrence, and comorbid conditions.

Definition of Analytic Variables

The 3-month interview served as the baseline time point for all subjects, and we restricted the sample to those who successfully completed a baseline interview (n = 689). We calculated the number of person-days of follow-up for each individual by extracting the number of days between the date of baseline interview and either the date of death or the date of last completed interview. Of the 689 subjects, 4.1% did not have recorded interview dates but had indicator variables for having completed an interview at each follow-up month. For these subjects, person-days were estimated by multiplying the number of months between surgery and last follow-up by 30.5 days. Eighty-seven subjects were lost to follow-up between the baseline and month 75 interviews; a further 203 were lost to follow-up after the month 75 interview.

Age at the time of primary treatment was divided into 3 categories for descriptive purposes (65–69, 70–79, and >79 years), but was modeled as a continuous variable. Race/ethnicity was self-reported as white, African American, Hispanic, Asian/Pacific Islander, Native American or Other. Regular exercise was defined in the interview question as "physical activity for at least one-half hour a day at least 3 times per week, with physical fitness being the main purpose of the activity," exclusive of any exercises prescribed by a subject's physician or physical therapist. We asked about exercise habits at the 6, 15, 27, 39, 51, and 87 month interviews.

We collected comorbidity data from participants at the 3, 27, 51, and 75 month interviews. We calculated the Charlson index of comorbidity,[14] using a method adapted to interview data instead of medical record abstractions.[15] Briefly, we constructed a Charlson score for each subject at each time point by assigning specified weights to 15 contributing health conditions if present at the time of interview. We translated the sum of the accrued weights into the ordinal Charlson index, which ranges from 0 (no comorbidity) to 3 (serious comorbidity). Once a subject reported a health condition it was assumed to persist for the remainder of a subject's follow-up time. Therefore, a given subject's Charlson index could either remain static or increase, but could not decrease over their follow-up time. A description of the ordinal Charlson index is given in Table 1.

The outcome for our study was death from any cause, ascertained by vital status queries of the National Death Index (NDI), the Social Security Administration (SSA), the death index of the Centers for Medicare and Medicaid Services (CMS), or by proxy interview response. We ascertained cause of death through regular queries of the NDI.

Selection of Candidate Confounders

We used a directed acyclic graph (DAG) to identify a sufficient set of confounders for analytic control. A DAG encodes hypothesized relations between variables, which can aid in identifying confounders of a given exposure-disease association. Confounders in a DAG are variables along a causal path with arrows pointing to both the exposure and disease (see Greenland et al[16] for a more complete definition). Figure 1 depicts the hypothesized relationships among the variables that influence comorbidity and all-cause mortality. Using the backdoor test described by Greenland et al,[16] control for age, exercise habits, and race/ethnicity were minimally sufficient to address confounding of the association between comorbidity and all-cause mortality, presuming the causal diagram faithfully depicts the causal relations among the variables. In our causal graph, tumor and treatment characteristics appear on the causal path-

TABLE 1. Formation of the Charlson Index

Charlson Weight	Comorbid Conditions
0	No comorbid conditions
1	Heart attack or treated for heart failure Surgical treatment for peripheral vascular disease Stroke, blood clot, or transient ischemic attack without loss of limb function Asthma treated with medications Peptic ulcer disease diagnosed by endoscopy or barium swallow Diabetes treated with oral medication or insulin injections Rheumatoid arthritis treated with medications, lupus, or polymyalgia rheumatica Alzheimer disease or other dementia
2	Stroke, blood clot, or transient ischemic attack with reduced arm or leg function Poor kidney function, high blood creatinine, ever used hemodialysis or peritoneal dialysis, or kidney transplant Diabetes with end-organ complications Diagnosed leukemia, lymphoma, or polycythemia vera
3	Cirrhosis or serious liver damage

Prevalent comorbid conditions for each subject were assigned weights according to the table. The sum of the weights was then used to form the ordinal Charlson index (Charlson index of 0, no comorbid conditions; Charlson index of 1, sum of weights equal to 1 or 2; Charlson index of 2, sum of weights equal to 3 or 4; Charlson index of 3, sum of weights >5).

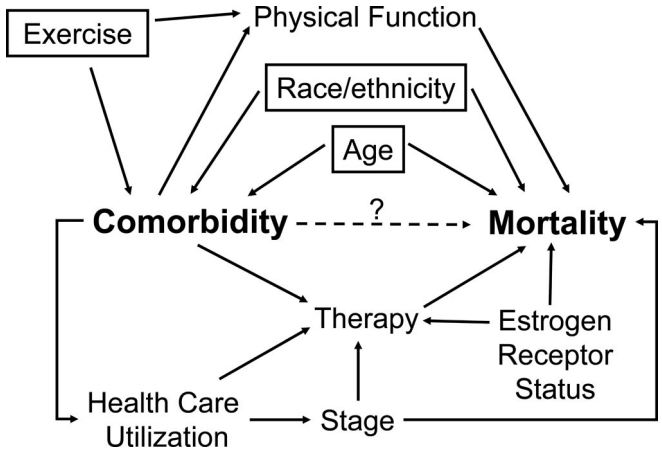

FIGURE 1. Directed acyclic graph depicting hypothesized relationships among covariates. Boxes indicate variables identified by backdoor test as confounders requiring adjustment. "Therapy" and "Stage" denote breast cancer treatment choices and AJCC disease staging, respectively.

way between comorbidity and all-cause mortality, making their control inappropriate. To do so would attenuate a portion of the total effect of comorbidity on all-cause mortality, leading to a biased measure of association.

Multiple Imputation of Missing Data

By design, all subjects had a baseline Charlson index. A considerable fraction of subjects (33%) had one or more missing values among their postbaseline Charlson index values, and 76% of subjects were missing one or more values among the 6 longitudinal exercise variables. To assess and correct for this loss to follow-up, we used a multiple imputation procedure to populate the missing data fields. A qualitative analysis of the data revealed nonmonotone patterns of missing values. That is, a missing value for either the Charlson index or exercise status at 1 time point did not always portend missing values at all future time points. Because of this nonmonotonicity, we were limited to using the Markov Chain Monte-Carlo (MCMC) imputation method. The MCMC method imputes continuous values for missing observations by drawing a specified number of fair random samples from a distribution characterized by the known values. Multiple imputation yields estimates of association that incorporate uncertainty about the imputed values into the variance of a parameter estimate, thus widening confidence intervals.[17] Five imputations were performed for each subject, using a single Markov chain and a noninformative prior distribution for the means and covariances of the missing Charlson and exercise data. Because the Charlson index is an ordinal measure, we constrained the range of imputed values between 0 and 3 and rounded to the nearest integer. The imputed dichotomous exercise variables were treated in an analogous manner.

To evaluate the performance of the multiple imputation procedure we selected a random sample of 20 subjects from those with complete Charlson data over the follow-up period (n = 462), recoded the subset's Charlson index values as missing, and imputed these values as described above. We compared the 5 imputed values at each follow-up point with the corresponding observed values and found that, overall, the imputed values matched the observed values 67% of the time. If the observed Charlson value was zero, the imputation matched 73% of the time, compared with a 56% match rate if the observed value was not equal to zero.

Statistical Analysis

We tabulated the number of subjects, cases of death, and person-days for the entire cohort based on sociodemographic, therapeutic, and comorbidity characteristics (Table 2). We modeled Cox proportional hazards to examine the effect of comorbidity on all-cause mortality when: (1) only baseline Charlson index was modeled; (2) the Charlson index was entered as a time-varying covariate, with missing values in the longitudinal scores addressed by carrying forward the last known observation; and (3) Charlson index was entered as a time-varying covariate, using imputed scores in place of the missing values. For the last of these procedures, 5 separate Cox models were obtained, one for each of the 5 imputations, and the results were combined to yield a single parameter estimate and standard error, accounting for the within- and between-imputation variability.[18] All models were additionally adjusted for age (continuous), race/ethnicity (nonwhite vs. white), and time-varying exercise habits (coded as a yes or no response to the regular exercise interview question). Models 1 and 2 were restricted to subjects who had nonmissing baseline exercise status (n = 612), and missing values for longitudinal exercise habits were

TABLE 2. Observed Baseline Characteristics of the Cohort (n = 689)

	No. Deaths	No. Subjects	Total Person-Days (%)
Age (yrs)			
65–69	33	176	395,769 (28)
70–79	112	383	822,089 (57)
>79	73	130	215,425 (15)
Race/ethnicity			
White	197	643	1,353,934 (94)
African American	15	34	58,903 (4.1)
Hispanic	1	3	5748 (0.4)
Asian/Pacific Islander	0	1	2669 (0.2)
Native American	1	1	916 (0.1)
Other	4	7	11,112 (0.8)
Baseline Charlson index			
0	93	390	850,230 (59)
1	95	248	485,639 (34)
2	21	38	77,758 (5.4)
3	9	13	19,586 (1.4)
Baseline exercise status			
Exercises regularly	83	350	767,999 (59)
Does not exercise regularly	96	262	542,221 (41)
Enrollment site			
Los Angeles	45	152	331,732 (23)
Rhode Island	64	174	355,070 (25)
Minnesota	65	190	384,647 (27)
North Carolina	44	173	361,834 (25)
AJCC* stage			
I	95	351	749,254 (52)
IIA	64	207	441,773 (31)
IIB	41	103	193,505 (14)
IIIA	17	27	48,101 (3.4)
Surgical treatment			
BCS, plus radiation therapy	46	218	496,136 (35)
BCS, no radiation therapy	42	111	220,916 (16)
Mastectomy	119	330	665,189 (47)
Other	9	19	31,292 (2.2)
Estrogen receptor status			
Positive	152	510	1,072,027 (76)
Negative	38	93	184,500 (13)
Unknown	25	76	152,627 (11)
Tamoxifen prescription			
Ever prescribed	135	453	959,096 (67)
Never prescribed	71	203	417,637 (29)
Unknown	12	33	56,550 (3.9)

*American Joint Committee on Cancer staging system.

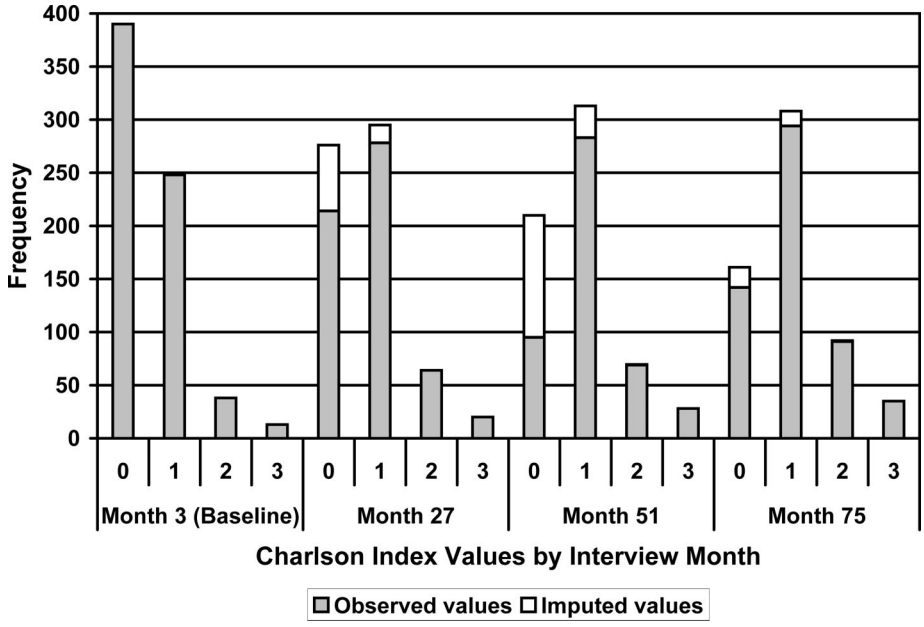

FIGURE 2. Distribution of observed and imputed Charlson index values by interview month following primary breast cancer surgery.

addressed by carrying forward the last known value. Imputed exercise status was used in Model 3.

The proportional hazards assumption for the Charlson index was verified for all 3 models by including a term for the interaction between Charlson index and the logarithm of person-days. The multiple imputation and statistical analyses were performed using SAS version 9 (SAS Institute, Cary, NC).

RESULTS

Six hundred eighty-nine subjects met the eligibility criteria and were included in the analysis. The total follow-up time was 3927 person-years, with a median individual follow-up time of 85 months. Table 2 displays the baseline characteristics of the analytic cohort. The majority of women in our study were white (95%), with a median age at enrollment of 73 years (range: 65–96 years). Most women (93%) began the study with a Charlson index of either 0 or 1, and 59% exercised regularly at baseline. Almost all of the participants (97%) had stage I or II breast cancer at diagnosis, and about half were treated with mastectomy. Of those who opted for breast-conserving surgery, only 66% received radiation therapy (RT).

Figure 2 shows the distribution of longitudinal Charlson index values before and after multiple imputation. Nearly all imputed Charlson index values were either 0 or 1, with little change in the proportion of moderate to severe comorbidity when combined with the measured values. The combined measured and imputed values demonstrate a trend toward more comorbidity over time, as expected.

Compared with the observed exercise values, the imputed values consistently showed a higher proportion of regular exercisers at each time point, but both sets showed an overall downward trend in the proportion of regular exercisers over time (data not shown).

Results from the 3 Cox models are shown in Table 3. Time-interaction terms for the Charlson index were nonsignificant in each of the 3 models ($P > 0.3$), thus verifying proportionality of hazards. The time-interaction terms were excluded from the final models. Each model result shows the relative increase in the hazard rate of death from any cause over our study's follow-up period (median: 85 months) associated with a 1-unit increase in the Charlson index. Model 1 considered only the baseline Charlson index while adjusting for age, race/ethnicity, and longitudinal exercise habits, with missing exercise values replaced by the last observation (HR: 1.4, 95% confidence interval [CI]: 1.2–1.7). Model 2 entered the Charlson index as a time-varying covariate, with missing

TABLE 3. Cox Proportional Hazards Models of All-Cause Mortality as a Function of Baseline or Acquired Comorbidity

Model	Parameter	Hazard Ratio*	95% CI	*P*
(1) Baseline Charlson	Charlson index	1.4	1.2–1.7	0.0004
	Age	2.4	1.9–3.0	<0.0001
	Race/ethnicity	1.6	1.0–2.7	0.06
	Exercise	1.1	0.9–1.3	0.19
(2) Time-varying Charlson index; missing values replaced by last known value	Charlson index	1.3	1.1–1.5	0.003
	Age	2.4	1.9–3.0	<0.0001
	Race/ethnicity	1.4	1.0–2.9	0.04
	Exercise	1.1	1.0–1.4	0.14
(3) Time-varying Charlson index; missing values multiply imputed†	Charlson index	1.4	1.2–1.6	0.0003
	Age	2.3	1.9–2.8	<0.0001
	Race/ethnicity	1.8	1.1–2.8	0.02
	Exercise	1.2	0.9–1.6	0.45

*Comparisons for hazard ratios; Charlson index, 1-unit increase in ordinal value; Age, 10-year increase; Race/ethnicity, nonwhite vs. white; Exercise, regular exercisers vs. nonregular exercisers.

†Missing values for exercise habits were also multiply imputed. Hazard ratio estimates and 95% confidence intervals were pooled over 5 imputations. *P* values are conservatively reported as the highest from among the 5 imputation models.

values replaced by the last known observation, adjusting for the same covariates as Model 1 (HR: 1.3, 95% CI: 1.1–1.5). Model 3 also considered acquired comorbidity but instead used multiply imputed Charlson index values and exercise status in place of missing longitudinal values (HR: 1.4, 95% CI: 1.2–1.6). We did not control for tumor and treatment characteristics because they are part of the causal pathway between comorbidity and mortality on our causal diagram (Fig. 1). Statistical adjustment for such variables would be expected to attenuate the observed hazard ratio by removing a portion of the total causal effect. We tested this expectation by additionally adjusting for stage, histologic grade, estrogen receptor status, surgery type, receipt of adjuvant tamoxifen, and receipt of adjuvant chemotherapy. Adjustment for these variables reduced the comorbidity hazard ratio in each of the 3 models by 5% or less.

DISCUSSION

We followed 689 breast cancer survivors for a median of 85 months; 33 months longer than a similar previous study.[8] We found that accounting only for baseline comorbidity gave approximately the same hazard ratio associating burden of comorbidity with all-cause mortality, compared with when comorbidity was regressed as a time-varying exposure (Hazard ratios: 1.4 and 1.3, respectively). Use of multiple imputation to populate missing values in longitudinal Charlson comorbidity data gave the same result (HR: 1.4). We consider our best estimate of the hazard ratio for a unit increase in Charlson index on the rate of all-cause mortality to be from Model 3, which used imputed values for all missing independent variables in the model. This estimate (HR: 1.4, 95% CI: 1.2–1.6) was consistent with the findings from an earlier study that examined the association between baseline Charlson index and all-cause mortality while controlling for age, primary treatment type, tumor stage, histologic grade, and hormone receptor status,[8] as well as a second study that examined only the impact of diabetes on all-cause mortality in breast cancer patients.[12]

Limitations

During our follow-up period, about 26% of subjects experienced an increase in comorbidity from baseline. Of those, approximately 80% had only a single-unit increase in Charlson index. These numbers indicate a relatively modest rate of comorbidity gain among cohort members. The present duration of follow-up—while the longest yet reported for a study of this association—may still be too short to capture an impact of longitudinal comorbidity on all-cause mortality rates. Our results indicate that a comorbidity assessment at the time of primary breast cancer treatment may provide sufficient short-term prognostic information (~7 years after surgery) for older breast cancer survivors.

Additional analyses with breast cancer mortality as the outcome would be of great interest. We could not conduct an appropriately powered analysis focused on breast cancer-specific mortality because the NDI registry does not yet contain cause of death data for all of the deceased subjects in our cohort.

The subjects in our cohort were predominately white (94%), so our results pertain mostly to women of that race. The poor representation of nonwhites in our cohort does not permit a rigorous evaluation of race/ethnicity as an effect modifier of the measured association between comorbidity and all-cause mortality.

Our results are susceptible to distortion by residual confounding, misclassification, and selection bias. Our exposure, outcome, and covariate data are subject to varying degrees of misclassification. Some subjects in our study were likely better historians of their medical history than others. There were 359 instances in which subjects failed to report one or more persisting medical condition in their 27, 51, and 87 month interviews, with respect to their baseline report. Of the 15 conditions that form the Charlson score, the most frequently under-reported at the 27-month interview—among those with a positive report for the condition at baseline—were heart failure (9%), diabetes (6%), stroke (5%), myocardial infarction (4%), connective tissue disease (4%), and pulmonary disease (3%). The remaining contributory conditions were under-reported with frequencies less than or equal to 2%. Under-reporting of medical history after the baseline interview was addressed by building monotonicity into the longitudinal Charlson index values. This method increased the sensitivity of comorbidity classification at the expense of specificity, which would cause overestimation of Charlson index if subjects falsely reported having certain conditions at any interview point. Although we cannot directly evaluate the extent of such overestimation in our own data, prior validation studies have shown that Charlson scores derived from interview data have test-retest reliability of approximately 0.9,[15] and are strongly correlated with scores derived from medical record review (correlation coefficient: 0.58, $P < 0.001$).[19] Our results did not differ substantially when we did not force monotonicity onto longitudinal Charlson index values, allowing them to decrease over time, indicating that our reported hazard ratios were not substantially affected by this potential source of misclassification of comorbidity.

Misclassification of confounders, if nondifferential with respect to outcome status, results in residual confounding.[20] Little, if any, misclassification is expected in the age and race/ethnicity variables, but exercise habits may be misreported by participants. Our measured exercise variable may also be an incomplete proxy for the conceptual entity for which we wished to control, which was routine physical activity that would affect the risk of both comorbid disease and all-cause mortality. The extent to which our measured exercise variable does not map to this concept informs the degree of residual confounding in our adjusted estimate of association. Adjustment for exercise decreased the crude hazard ratio associated with Charlson index by 2%, indicating a slight bias away from the null due to confounding by exercise habits. If exercise was nondifferentially misclassified in our data, 2% would be an underestimate of the true magnitude of the upward confounding bias and the true adjusted hazard ratio would be lower than what we observed. Validation studies of specific physical activity instruments have shown significant correlations between older subjects'

responses and objectively measured physiologic parameters, indicating regular physical activity[21,22] as well as with results from "gold standard" doubly-labeled water experiments.[23] Although our assessment of exercise habits relied on none of the particular instruments examined by the validation studies, our question to participants was detailed and specific in nature and should be of similar validity. Responses to this interview question are expected to conform reasonably well to the ideal concept for which we sought to adjust. We therefore do not expect residual confounding by exercise to be of a sufficient magnitude to explain our result completely.

Misclassification of vital status is unlikely, given the reliability of the National Death Index, Social Security Administration, and Centers for Medicare and Medicaid Services death indices. Approximately 94% of deaths in our cohort were ascertained from the NDI, which has consistently demonstrated high sensitivity and specificity (both nearly 100%) for vital status.[20,24] Approximately 5% of deaths were ascertained through the Social Security Administration database, which while inferior to the NDI, also exhibits favorable classification accuracy.[24] Proxy interviews and the CMS database contributed only 1 death each, and any flaws in these sources would not have substantially influenced our results.

We restricted our analytic sample to women in our cohort who had completed a baseline interview (3 months after primary breast cancer surgery). If completion of the baseline interview was an effect of both comorbidity (or its absence) and vital status, then selection bias could distort our observed association.[25] We believe the most likely scenario is that subjects with a greater comorbidity burden at enrollment were less likely to complete their 3-month interview, either because of their illness or because of death before the 3-month point. If this pattern is indeed the case, the selection bias would have the effect of lowering the observed association between comorbidity and all-cause mortality, and could not account for our result. In support of this pattern, we observed a 42% higher odds of dying among the cohort subjects who did not complete a baseline interview, compared with those who did (OR: 1.42, 95% CI: 1.00–2.00). Our cohort also experienced loss to follow-up, which resulted in missing data in the exposure (comorbidity) and confounder (exercise) data. The sensitivity of our observed results to these losses was tested by modeling with multiply imputed values replacing the missing fields. Multiple imputation is an attractive alternative to carrying forward prior observations in longitudinal analyses; it yields results that incorporate uncertainty about the imputed values into confidence intervals.[17] Examination of our survival analysis results (Table 3) shows that the confidence interval around the hazard ratio corresponding to the multiply imputed data is actually the narrowest, despite the additional uncertainty it contains. This counter-intuitive result is likely explained by the ability of this model to include 71 additional observations from subjects without baseline exercise data. Inclusion of these observations in the imputation model apparently increases precision more than the imputation decreases it.

In conclusion, we found that a unit increase in the Charlson index of comorbidity was associated with a 40% higher hazard of death from any cause among older survivors of early stage female breast cancer. The same general result was observed whether or not we accounted for acquired comorbidities and missing data. The modest rate of comorbidity gain in our cohort may be responsible for the equivalent results between longitudinal and baseline-only accounting of comorbidity. Additional prognostic value of longitudinal comorbidity may become evident upon longer follow-up.

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Research article

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Digoxin treatment is associated with an increased incidence of breast cancer: a population-based case-control study

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Abstract

Introduction Laboratory and epidemiologic studies have suggested a modifying effect of cardiac glycosides (for example, digoxin and digitoxin) on cancer risk. We explored the association between digoxin treatment and invasive breast cancer incidence among postmenopausal Danish women.

Methods We used Danish registries to identify 5,565 postmenopausal women diagnosed with incident invasive breast carcinoma between 1 January 1991 and 31 December 2007, and 55,650 matched population controls. Cardiac glycoside prescriptions were ascertained from county prescription registries. All subjects had at least 2 years of recorded prescription drug and medical history data. We estimated the odds ratio associating digoxin use with breast cancer in conditional logistic regression models adjusted for age, county of residence, and use of anticoagulants, non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, and hormone replacement therapy. We also explored the impact of confounding by indication and detection bias.

Results Digoxin was the sole cardiac glycoside prescribed to subjects during the study period. There were 324 breast cancer cases (5.8%) and 2,546 controls (4.6%) with a history of digoxin use at least 1 year before their index date (adjusted odds ratio (OR): 1.30; 95% confidence interval: 1.14 to 1.48). The breast cancer OR increased modestly with increasing duration of digoxin exposure (adjusted OR for 7 to 18 years of digoxin use: 1.39; 95% confidence interval: 1.10 to 1.74). The association was robust to adjustment for age, receipt of hormone replacement therapy, coprescribed drugs, and confounding by indication. A comparison of screening mammography rates between cases and controls showed no evidence of detection bias.

Conclusions Our results suggest that digoxin treatment increases the risk of invasive breast cancer among postmenopausal women.

Introduction

Cardiac glycosides (CGs) are natural steroid toxins that have been used since the 18th century to treat congestive heart failure (CHF) and atrial fibrillation (AF) [1]. The clinically most prevalent CGs are the *Digitalis*-derived cardenolides digitoxin and digoxin. These compounds exert their pharmacologic effect via inhibition of the Na^+/K^+ ATPase, which indirectly raises intracellular Ca^{2+} concentration, thus increasing the force of contractility in cardiac myocytes.

In 1979, Stenkvist *et al.* reported an unusual finding in a small cohort of breast cancer patients (n = 142) [2]. Women in the cohort who were taking CGs (mostly digoxin) at the time of their breast cancer diagnosis had tumors with less aggressive phenotypes than breast tumors of women not taking CGs [2]. They later reported a higher recurrence rate among the women not taking CGs after 5 [3] and approximately 22 [4] years of follow-up. These observations suggested a beneficial effect of cardiac glycosides for women with breast tumors. An early mechanistic hypothesis centered on CG interference with estrogen receptor (ER) signaling in tumor cells [2], while current laboratory studies implicate novel signaling pathways mediated by the Na^+/K^+ ATPase [5,6].

AF: atrial fibrillation; BMI: body mass index; CG: cardiac glycoside; CHF: congestive heart failure; CI: confidence interval; CVD: cardiovascular disease; ER: estrogen receptor; HRT: hormone replacement therapy; ICD: International Classification of Diseases; OR: odds ratio; SIR: standardized incidence ratio.

Subsequent studies of the association between CG use and breast cancer incidence gave conflicting results. Haux *et al*. compared site-specific cancer incidence rates among digitalis-treated Norwegians patients with expected rates in the general population [7]. Several cancers, including female breast cancer, occurred at higher rates among those treated with digitalis compared with the general population [7]. Also, Friedman reported no association between CG prescription history and breast cancer in a Kaiser-Permanente registry study [8].

Given the continued importance of CG medicines to treat heart disease and the inconsistent results from earlier studies of the association between this therapy and breast cancer occurrence, we examined the association between digoxin treatment and breast tumor incidence rate in a population-based prospective case-control study of postmenopausal Danish women.

Materials and methods

This study was approved by the Boston University Medical Campus Institutional Review Board and the Danish Registry Board.

Study population

This study was conducted within the female population of North Jutland and Aarhus Counties, Denmark [9]. We used county hospital registries to ascertain all cases of incident invasive breast cancer diagnosed in women age 55 or older. Ascertainment began on 1 January 1991 in North Jutland County and 1 January 1998 in Aarhus County, and continued until 31 December 2007 [10]. The hospital registries contain data on patients' civil personal registry (CPR) number, date(s) of admission, date(s) of discharge, and up to 20 discharge diagnoses and medical procedures per discharge or outpatient visit. Diagnoses are assigned by the attending physician, and are coded according to the *International Classification of Diseases*, 8th revision (ICD-8, until 1995) and 10th revision (ICD-10, 1995 onwards).

Controls were identified in the Danish Civil Registration System, which has tracked residential address, vital status, and date of emigration for the entire Danish population since 1968 [11]. Controls were selected for each case by risk-set sampling, matching controls to cases on year of birth and county of residence. Within strata of the matching factors, we selected 10 controls at random among those who were alive and without a history of breast cancer on the date of the matched case's diagnosis. This date was the index date for the cases and matched controls.

Data collection

We used each subject's unique CPR number to link the case-control roster to county prescription databases [12,13], which automatically record all prescriptions filled since 1989 in North Jutland County and 1996 in Aarhus County. The databases encode drugs by the Anatomical Therapeutic Chemical (ATC) classification system [14] and record dates of all prescription fills along with the patient's CPR number. These systems report prescription data to the county databases, as well as to the Danish National Health Service, which refunds a portion of medication costs. Prescriptions are logged in the registries after patients present to a pharmacy and pay their share of the prescription cost. To ensure adequate prescription data history, we excluded cases and controls who had lived in the study counties for less than 2 years after the establishment of electronic prescription registries. We ascertained medical history for cases and controls by extracting major diagnoses preceding index dates from the county hospital registries. We also used these registries to identify all prediagnosis mammography procedures for cases and controls since 2001, the year mammography data began to be systematically recorded.

Definitions of analytic variables

We identified cases of incident breast cancer in the hospital registries using ICD-8 and ICD-10 codes appropriate to the date ranges of the databases. ICD codes were also used to ascertain comorbid conditions for cases and controls (see full ICD code listing in Table 1).

We ascertained CG prescriptions by extracting all records from the prescription databases with ATC codes beginning with C01A. CGs are available only by prescription in Denmark, and are dispensed at pharmacies equipped with automated electronic reporting systems described in the data collection section. This strategy captured all CG prescriptions in the counties over the study period that were for digoxin exclusively. Digoxin prescriptions were only considered if they occurred at least 1 year before the index date. Digoxin exposure was considered in broad terms as ever exposed ($\geq$ 1 digoxin prescription at least 1 year before the index date) or never exposed (no record of digoxin prescription at least 1 year before the index date), and in finer terms according to the length of time between a woman's first digoxin prescription and her index date.

Confounders were selected *a priori* based upon established breast cancer risk factors that were also likely to influence receipt of digoxin. Age was initially controlled by matching cases to controls on year of birth. We also calculated each subject's exact age on her index date to adjust for residual confounding by age. We additionally considered confounding by coprescription of anticoagulants, non-steroidal anti-inflammatory drugs (NSAIDs), aspirin, and hormone replacement therapy (HRT). Anticoagulants are frequently prescribed for AF, and were associated with lower risk of urogenital cancer [15]. NSAID use has been associated with increased risk of CHF [16], and these drugs have shown protective associations with breast cancer in some studies [17]. Aspirin use, which may be more prevalent among digoxin users, has been

Table 1

Listing of ICD-8 and ICD-10 codes used to ascertain key diagnoses

Diagnosis	ICD-8	ICD-10
Invasive breast carcinoma[a]	174.00 to 174.02; 174.08; 174.09;	C50.0 to C50.6; C50.8; C50.9
Congestive heart failure	427.09; 427.10; 427.11; 427.19; 428.99; 782.49	I50; I11.0; I13.0; I13.2
Atrial fibrillation/flutter[b]	427.93; 427.94	I48
Myocardial infarction	410	I21; I22; I23
Peripheral vascular disease	440; 441; 442; 443; 444; 445	I70; I71; I72; I73; I74; I77
Cerebrovascular disease	430 to 438	I60 to I69; G45; G46
Chronic pulmonary disease	490 to 493; 515 to 518	J40 to J47; J60 to J67; J68.4; J70.1; J70.3; J84.1; J92.0; J96.1; J98.2; J98.3
Mild liver disease	571; 573.01; 573.04	B18; K70.0 to K70.3; K70.9; K71; K73; K74; K76.0
Moderate to severe liver disease	070.00; 070.02; 070.04; 070.06; 070.08; 573.00; 456.00 to 456.09	B15.0; B16.0; B16.2; B19.0; K70.4; K72; K76.6; I85
Diabetes type 1	249.00; 249.06; 249.07; 249.09	E10.0, E10.1; E10.9
Diabetes type 2	250.00; 250.06; 250.07; 250.09	E11.0; E11.1; E11.9
Moderate to severe renal disease	403; 404; 580 to 583; 584; 590.09; 593.19; 753.10 to 753.19; 792	I12; I13; N00 to N05; N07; N11; N14; N17 to N19; Q61
Diabetes with end organ damage (types 1 and 2)	249.01 to 249.05; 249.08; 250.01 to 250.05; 250.08	E10.2 to E10.8; E11.2 to E11.8
Solid tumor	140 to 194	C00 to C75
Lymphoma	200 to 203; 275.59	C81 to C85; C88; C90; C96

[a]The ICD codes for invasive breast carcinoma do not capture *in situ* tumors (for example, intraductal carcinoma); [b]ICD-8 contained separate codes for atrial fibrillation (427.93) and flutter (427.94). These two diagnoses were combined into a single code in ICD-10 (I48).
ICD, International Classification of Disease.

associated with reduced breast cancer risk [18], though data are conflicting [17]. We therefore evaluated confounding by low- and high-dose aspirin use. We also evaluated HRT as a confounder because of its contribution to cumulative hormonal exposure and its association with breast cancer risk [19].

Prescriptions for hormone replacement therapy were identified by ATC codes (estrogens: codes starting with either G03C or L02AA; progestin: codes beginning with G03D; combination therapy: codes beginning with either G03F or G03H). Exposure to any of these drugs before the index date was classified as 'ever exposed to HRT' while exposure to none of them was classified as 'never exposed to HRT'. Similarly, we characterized ever/never exposure to anticoagulants, NSAIDs and aspirin by searching for ATC codes beginning with B01A, M01A, and B01AC06, respectively.

We evaluated confounding by the medical indications for digoxin therapy by defining an alternative reference group of women who were never exposed to digoxin and who had a history of cardiovascular disease (excluding CHF or AF). We hypothesized these reference subjects should be more similar to the digoxin-treated women with regard to cumulative hormonal exposures and lifestyle factors that may modify risk for both heart disease and breast cancer. This reference group also facilitated evaluation of detection bias by allowing comparison of digoxin-exposed women to women with other serious histories who would likely have similar medical usage patterns. We further evaluated detection bias by comparing mammography usage rates between cases and controls. Dates of all mammography procedures among cases and controls were identified in hospital registries using appropriate Danish medical procedure codes. We analyzed mammography usage among women with index dates from 1 January 2006 onward, the period of our study when screening mammography would have been most common in Denmark. For each subject who had undergone mammography before her index date, we identified her most recent procedure and calculated the time elapsed between that procedure and the index date.

Statistical analysis

We characterized the names, doses, and prescribing frequencies of the various digoxin products used over the study period. We computed the frequency and proportion of cases and controls by digoxin exposure status, prevalent medical

conditions, use of other prescription drugs (HRT, anticoagulants, NSAIDs and aspirin), and age on index date.

We calculated the unadjusted odds ratios (ORs) and 95% confidence intervals (CIs) associating digoxin exposure categories with incident breast cancer and used conditional logistic regression to account for the matching factors and to adjust for exact age and past use of HRT, anticoagulants, NSAIDs, and aspirin. Due to the risk-set sampling design, the odds ratio approximates the incidence rate ratio associating digoxin exposure with incident breast cancer [20]. All analyses were performed with SAS version 9 (SAS Institute, Cary, NC, USA).

Results

Characteristics of cases and controls

We identified 5,565 cases and 55,650 matched population controls. Among the cases, 324 (5.8%) had ever had a digoxin prescription at least 1 year before her diagnosis date and 2,546 (4.6%) of controls had ever had a digoxin prescription at least 1 year before her index date. The distributions of cases and controls according to age, mammography usage, comorbidity and relevant prescription drug usage are shown in Table 2. By virtue of the matching, cases and controls were identical with respect to age distribution. Cases were somewhat more likely to have CHF, AF, chronic pulmonary disease, or diabetes, and were less likely to have a history of myocardial infarction, than controls. Cases also had more exposure to HRT, anticoagulants and NSAIDs than controls. As expected, mammography usage was substantially higher for cases than for controls in the year preceding the index date (81% vs 1.6%, respectively). However, usage was similar for cases and controls in time periods more distant from index dates.

Digoxin treatment and incident breast cancer

Table 3 shows all of the cardiac glycoside products recorded in the county prescription registries during the study period. We noted that digoxin was the sole CG used during this period. Approximately 97% of all digoxin prescriptions were for 62.5 μg tablets, indicating very little product heterogeneity among the digoxin-exposed subjects.

We observed a higher rate of breast cancer among ever-users of digoxin, relative to never users, in both unadjusted and adjusted analyses (adjusted OR: 1.30; 95% CI: 1.14 to 1.48; Table 4). This association persisted in categories of drug exposure duration (1 to 3 years, 4 to 6 years and 7 to 18 years), with a suggested upward trend in the odds ratios with increasing duration of digoxin therapy. When we compared digoxin-exposed women with the alternative reference group of unexposed women with cardiovascular medical histories, we continued to observe an association between digoxin exposure and incident breast cancer (adjusted OR: 1.42; 95% CI: 1.14 to 1.77).

Discussion

Our results suggest there may be a causal association between digoxin treatment and incident breast cancer in postmenopausal women. These findings were robust to adjustment for key confounders, confounding by indication, and medical detection bias.

Interestingly, results from a case-control study by Stenkvist *et al*. agree with our present findings. The investigators compared the CG exposure history of the breast cancer cases from their original report [2] to the exposure history of age-matched controls from the general population [21]. The authors concluded that CGs had no influence on breast cancer incidence, due to a non-significant chi-squared test for independence ($p = 0.25$). The data from the published cross-tabulation in fact yield an OR of 1.39, with a 95% CI of 0.79 to 2.45. While the interval is somewhat wide, the OR is near to our result and consistent with a causal association between CG use and incident breast cancer.

Other previous research is consistent with our results [7,22]. Haux and colleagues observed an elevated breast cancer rate (standardized incidence ratio (SIR): 1.25; 95% CI: 0.95 to 1.62) among mostly postmenopausal digitoxin users, compared with the rate in the general population [7]. The authors also observed elevated SIR for several other cancer sites [7]. Friedman reported results from a Kaiser Permanente cohort study of carcinogenic effects of prescription drugs, which showed no statistically significant association between digitalis treatment and breast cancer incidence. However, the SIR for this association was 1.2 – similar to the result of our study. Ewertz *et al*. found a positive association between digoxin usage and incident male breast cancer (OR for = 5 years of digoxin use: 2.0; 95% CI: 0.9 to 4.4) [22]. Together these results argue against ER antagonism by digitalis glycosides. Our results are more consistent with an ER agonist property of digoxin, though some *in vitro* ER binding studies do not support this notion [23,24].

Recent laboratory findings implicate the Na^+/K^+ ATPase in a variety of signal transduction pathways, with end effects in cell adhesion, survival, and proliferation [25]. Several *in vitro* studies point toward a downstream antiproliferative effect of CGs but others leave open the possibility of cancer-promoting endpoints [26]. The interaction of cardiac glycosides with the Na^+/K^+ ATPase and the consequential effects appear to be highly dependent on the specific CG compound and the subunit makeup of the receiving ATPase [6,26,27]. Therefore it would not be surprising to observe inconsistent responses of different human tissue types to the diverse cardiac glycosides. Some of these ligand-, receptor-, and tissue-specific responses may plausibly result in breast tumorigenesis *in vivo*, consistent with our findings. With this study, we have isolated the association between a single cardiac glycoside, digoxin,

Table 2

Characteristics of the study sample

Variable	Cases (n = 5,565)	Controls (n = 55,650)
Age on index date (years):		
55 to 64	2,116 (38)	21,160 (38)
65 to 74	1,800 (32)	18,000 (32)
75 to 84	1,356 (24)	13,560 (24)
≥ 85	293 (5.3)	2,930 (5.3)
Medical history, n (%):		
Congestive heart failure	160 (2.9)	1,337 (2.4)
Atrial fibrillation/flutter	224 (4.0)	1,819 (3.3)
Prediagnosis mammography[a]		
< 1 year:	417 (81)	84 (1.6)
1 to < 2 years:	3 (0.6)	84 (1.6)
2 to < 3 years:	9 (1.7)	84 (1.6)
≥ 3 years:	17 (3.3)	130 (2.5)
Myocardial infarction	123 (2.2)	1,492 (2.7)
Chronic pulmonary disease	334 (6.0)	3,125 (5.6)
Peripheral vascular disease	167 (3.0)	1,563 (2.8)
Cerebrovascular disease	275 (4.9)	2,842 (5.1)
Lymphoma	12 (0.2)	155 (0.3)
Other solid tumor	0	0
Liver disease	44 (0.8)	403 (0.7)
Diabetes (type I or II)	215 (3.9)	1,706 (3.1)
Diabetes with end-organ complication	85 (1.5)	591 (1.1)
Renal disease	35 (0.6)	446 (0.8)
Other drug exposures, n (%):		
Hormone replacement therapy	2,062 (37)	17,582 (32)
Anticoagulants	231 (4.2)	2,109 (3.8)
NSAIDs	3,106 (56)	29,964 (54)
Aspirin, low-dose (< 150 mg)	205 (3.7)	2,004 (3.6)
Aspirin, high-dose (≥ 150 mg)	505 (9.1)	4,878 (8.8)

[a]Screening mammography data were only available from 2001 onwards. We restricted the mammography analysis to cases and controls with index dates after 1 January 2006, when screening mammography would have been most common in Denmark. Categories reflect time elapsed between most recent mammogram and index date; proportion denominators are the total number of cases (n = 516) or controls (n = 5,160) in the restricted data set.
NSAID, non-steroidal anti-inflammatory drug.

Table 3

All cardiac glycoside products prescribed to study subjects[a]

Product name	Dose	Fill quantity	No. of prescriptions, (% of total)
Digoxin	62.5 μg/tablet	100 tablets	83,094 (66)
	62.5 μg/tablet	200 tablets	38,188 (31)
	250 μg/tablet	100 tablets	4,047 (3.2)
	50 μg/mL	30 mL	28 (0.02)

[a]Result of searching the prescription database for all Anatomical Therapeutic Chemical (ATC) codes beginning with 'C01A'.

and breast cancer incidence in a virtually unselected population of postmenopausal women.

Strengths and limitations

The main strengths of this study are its large size, use of high-validity registry data to ascertain diagnoses, use of prospectively-recorded exposure information, and lack of selection in enumerating cases and controls.

Our study design minimized the threat of selection bias, which can create the illusion of an exposure-disease association when, in fact, none exists [28]. We had only one subject exclusion criterion, and controls were selected completely at random within strata of the matching factors. Since no subject was required to give their consent to participate, no self-selection mechanism could have influenced our results.

Our results are subject to distortion by residual confounding and misclassification of exposure and outcome. We took measures to address confounding by age, past exposure to other prescription drugs, and the medical indications for digoxin prescription. We saw little change in the unadjusted association after accounting for these factors. Digoxin is ordinarily prescribed at an age when most women no longer bear children, so it is unlikely that digoxin exposure is strongly associated with the well-characterized reproductive factors that affect breast cancer risk [29]. We therefore do not expect substantial residual confounding. It is unlikely that use of other prescription drugs could bias our results, since antibiotics,

Table 4

Associations between digoxin treatment and incident breast cancer

Exposure categories	Cases (n = 5,565)	Controls (n = 55,650)	Unadjusted OR (95% CI)	Adjusted[a] OR (95% CI)
Ever/never prescribed digoxin:				
Ever user	324	2,546	1.29 (1.14 to 1.45)	1.30 (1.14 to 1.48)
Never user	5,241	53,104	1.0 (Ref)	1.0 (Ref)
Duration of digoxin therapy:[b]				
7 to 18 years	93	694	1.35 (1.10 to 1.69)	1.39 (1.10 to 1.74)
4 to 6 years	103	811	1.29 (1.05 to 1.58)	1.30 (1.05 to 1.61)
1 to 3 years	128	1,041	1.25 (1.03 to 1.50)	1.25 (1.03 to 1.52)
Never user	5,241	53,104	1.0 (Ref)	1.0 (Ref)
Ever/never prescribed digoxin (alternate reference group):	(n = 732)	(n = 7,086)		
Ever user	324	2,546	1.42 (1.21 to 1.65)	1.42 (1.14 to 1.77)
Never user[c]	408	4,540	1.0 (Ref)	1.0 (Ref)

[a]Adjusted for age (continuous), county of residence (categorical), and past receipt of hormone replacement therapy, anticoagulants, high- and low-dose aspirin, and non-steroidal anti-inflammatory drugs (NSAIDs) (ever/never); [b]years elapsed between first digoxin prescription and index date (approximate tertiles of the distribution); [c]the alternate reference group is additionally defined by a history of myocardial infarction, peripheral vascular disease, cerebrovascular disease, or any combination thereof. See text for rationale.
CI, confidence interval; OR, odds ratio.

antihypertensives, statins, and antidepressants do not appear to modify breast cancer risk [17]. Use of the alternative reference group resulted in a modest increase in the estimated odds ratio; this result implies that confounding by indication actually served to attenuate the original association. Furthermore, detection bias is not likely to account for the observed association, since women with other cardiovascular diseases would have similar medical usage to women treated with CG. In the whole study population, we saw no material difference in mammography usage rates between cases and controls in time periods distant from index dates, which further argues against detection bias.

We were not able to adjust directly for body mass index (BMI), which is associated with both cardiovascular disease (CVD) and breast cancer [30]. However our alternative reference group likely controlled in part for BMI due to the association of BMI with CVD [31]. Since the effect of adjustment via this reference group was to move the odds ratio estimate away from the null, it is unlikely that unmeasured confounding by BMI could account for our positive result.

Our characterization of digoxin exposure was informed only by the number and strength of prescriptions filled by study participants; the prescription registry data did not permit calculation of actual daily doses taken by exposed subjects. Because prescription records were generated automatically before breast cancer diagnoses, we expect any exposure classification error to be non-differential in nature. We are not aware of published validation data on the classification of incident breast cancer in the hospital discharge registries. However, breast cancer diagnoses were recorded without express knowledge of exposure, so outcome misclassification is also expected to be non-differential. Since non-differential classification errors are expected to attenuate results, exposure and outcome misclassification cannot plausibly account for our positive association [28].

Conclusion

We observed a modestly increased rate of breast cancer among postmenopausal women with any history of digoxin use, compared with women with no such use, after adjustment for age, use of other prescription drugs, and cardiovascular indications. The associations persisted in long-term exposure categories. While a number of laboratory studies of cardiac glycosides and female breast cancer have suggested protective effects, our results suggest that one specific cardiac glycoside, digoxin, moderately increases the incidence rate of breast cancer. This finding agrees with results from past studies; [7,8,21] the importance of which were likely masked by large standard errors of the association measures.

Competing interests

HTS reports receiving no fees, honoraria, grants or consultancies. The Department of Clinical Epidemiology is, however, involved in studies with funding from various pharmaceutical companies (Amgen, Pfizer, Glaxo and Centocor) as research grants to (and administered by) Aarhus University. None of these studies have relation to the present study. TPA, LAP and TLL declare no conflicts of interest.

Authors' contributions

HTS, TPA and TLL conceived the study idea. TPA, TLL and HTS designed the study. HTS and LAP collected the data. TPA performed all data analyses, reviewed the literature and wrote the first draft of the manuscript. All authors edited the manuscript.

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Full Paper

Tamoxifen's protection against breast cancer recurrence is not reduced by concurrent use of the SSRI citalopram

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Tamoxifen remains an important adjuvant therapy to reduce the rate of breast cancer recurrence among patients with oestrogen-receptor-positive tumours. Cytochrome P-450 2D6 metabolises tamoxifen to metabolites that more readily bind the oestrogen receptor. This enzyme also metabolises selective serotonin reuptake inhibitors (SSRI), so these widely used drugs – when taken concurrently – may reduce tamoxifen's prevention of breast cancer recurrence. We studied citalopram use in 184 cases of breast cancer recurrence and 184 matched controls without recurrence after equivalent follow-up. Cases and controls were nested in a population of female residents of Northern Denmark with stages I–III oestrogen-receptor-positive breast cancer 1985–2001 and who took tamoxifen for 1, 2, or most often for 5 years. We ascertained prescription histories by linking participants' central personal registry numbers to prescription databases from the National Health Service. Seventeen cases (9%) and 21 controls (11%) received at least one prescription for the SSRI citalopram while taking tamoxifen (adjusted conditional odds ratio = 0.85, 95% confidence interval = 0.42, 1.7). We also observed no reduction of tamoxifen effectiveness among regular citalopram users (⩾30% overlap with tamoxifen use). These results suggest that concurrent use of citalopram does not reduce tamoxifen's prevention of breast cancer recurrence.

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Tamoxifen is a selective oestrogen receptor modulator (Jordan and Dowse, 1976) that reduces by half the risk of breast cancer recurrence in early-stage patients whose tumour cells express the oestrogen receptor (Early Breast Cancer Trialists' Collaborative Group, 2005). To be pharmacologically active, tamoxifen must be metabolised to secondary metabolites that bind the oestrogen receptor 100-fold more readily than tamoxifen itself (Malet *et al*, 1988). Four cytochrome P-450 enzymes (CYPs) catalyse this activation (CYP2D6, CYP3A4, CYP3A5, and CYP2C9) (Malet *et al*, 1988). CYP2D6 catalyses formation of 4-hydroxytamoxifen from tamoxifen (Coller *et al*, 2002) and formation of 4-hydroxy-*N*-desmethyltamoxifen from *N*-desmethyltamoxifen (Stearns *et al*, 2003). These two secondary metabolites have the highest binding affinity for the oestrogen receptor, and binding affinity correlates with inhibition of cell growth (Coezy *et al*, 1982). The secondary metabolites are, therefore, the most important modulators of the oestrogen receptor in the tamoxifen pathway (Lim *et al*, 2005).

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Breast cancer patients treated with tamoxifen may also take other prescription medications that are metabolised by some of the same enzymes that activate tamoxifen. For example, depression is a common comorbidity in breast cancer patients (Massie, 2004), and many selective serotonin reuptake inhibitors (SSRI), which are widely used medications indicated primarily to treat depression (Hansen *et al*, 2003), are metabolised by CYP2D6 (Zanger *et al*, 2004). SSRI competition with tamoxifen and *N*-desmethyltamoxifen for CYP2D6, or direct inhibition of CYP2D6 by SSRI, could reduce the production of the tamoxifen metabolites with high receptor-binding affinity, and thereby reduce tamoxifen's prevention of breast cancer recurrence. Competition between tamoxifen and the SSRI paroxetine reduced the plasma concentration of endoxifen in a cross-over clinical trial (Stearns *et al*, 2003). Furthermore, the mean plasma concentration of 4-hydroxy-*N*-desmethyltamoxifen was more than two-fold greater among women who were taking no CYP2D6 competitor drug than among women who were taking such a drug (Jin *et al*, 2005). *In vivo* studies thus demonstrate a compelling biological basis for the hypothesis that concomitant use of SSRI would reduce tamoxifen's prevention of breast cancer recurrence.

In the largest study to date of the potential for drug–drug interaction to reduce tamoxifen's protection against breast cancer

recurrence, we examined whether Danish breast cancer patients with oestrogen-receptor-positive tumours who were treated with tamoxifen for 1, 2, or most often for 5 years had a higher rate of recurrence if they were concomitantly taking the SSRI citalopram or its S-stereoisomer ('citalopram' from here onwards) than if they were not. As described in more detail below, citalopram was the most frequently prescribed SSRI in the study population.

MATERIALS AND METHODS

The study was approved by the Boston University Medical Campus Institutional Review Board and The Regional Committee on Biomedical Research Ethics of Aarhus County.

Study population

The source population included female residents of four Northern Danish counties (Aarhus, North Jutland, Viborg, and Ringkøbing) aged 35–69 at diagnosis of primary International Union Against Cancer stage I, II, or III breast cancer (UICC, 1997) between 1985 and 2001 and who were reported to the Danish Breast Cancer Cooperative Group (DBCG). The DBCG has enrolled nearly all Danish breast cancer patients younger than age 70 at diagnosis into its clinical database since 1977 (Andersen and Mouridsen, 1988; Jensen *et al*, 2003). More than 90% of Danish breast cancer cases are reported to the DBCG and more than half of the DBCG patients are enrolled in clinical trials (Andersen and Mouridsen, 1988). The same standardized forms are used to follow all patients reported to the DBCG, regardless of whether they enrol in a trial, so the registry provides the data quality advantage of a clinical trial setting with the generalisability advantage of a population-based setting.

We divided the source population into three groups: (a) group I women whose tumour expressed the oestrogen receptor protein and who were treated with tamoxifen for at least 1 year; (b) group II women whose tumour did not express the oestrogen receptor protein, were not treated with tamoxifen, and who survived for at least one year; and (c) group III women, comprising all others, who were excluded from this analysis. Group I women were assigned to tamoxifen therapy protocols of 1, 2, or 5 years, depending on the guideline extant in Denmark at the time of their diagnoses. We included group II women to estimate the direct association of citalopram prescription with recurrence rate, if any. We further restricted the source population to women diagnosed with breast cancer after the date that their county of residence began to maintain an electronic prescription database (Aarhus = 1996, North Jutland = 1989, Ringkøbing = 1998, Viborg = 1998), which were used to ascertain use of prescription medications, including citalopram. Follow-up time began 1 year after the date of breast cancer diagnosis and continued until the date of the first of breast cancer recurrence, death from any cause, loss to follow-up (e.g., emigration), 10 years of follow-up, or 1 September 2006.

Cases were women with local or distant breast cancer recurrence occurring during their follow-up time among the members of groups I and II. We selected one control for each case without replacement from members of the source population who had not had a breast cancer recurrence after the same amount of follow-up time. We matched controls to cases on (a) group membership (group I or II), (b) menopausal status at diagnosis (premenopausal or postmenopausal), (c) date of breast cancer surgery (caliper matched ± 12 months), (d) county of residence at the time of diagnosis, and (e) UICC stage at diagnosis (stage I, II, or III).

Data collection

We used the Danish Civil Personal Registration (CPR) number assigned to each case and control to link data sets. The CPR is a unique identification number assigned to all Danish residents alive on 1 April 1968, born thereafter, or upon immigration.

We collected demographic information (age, menopausal status, and hospital of diagnosis), tumour characteristics (UICC stage, histological grade, and oestrogen-receptor expression), and therapy characteristics (primary surgical tumour management, receipt of radiation therapy, receipt of chemotherapy, and receipt of tamoxifen therapy) from the DBCG database.

We collected data on receipt of citalopram prescription and other potential CYP2D6 inhibitors (including other SSRI) by linking the CPR number of cases and controls to the prescription databases maintained by each county (see, for example, the description of North Jutland's database (Gaist *et al*, 1997)).

Analytic variables

Recurrence We used the DBCG definition of breast cancer recurrence as any type of breast cancer subsequent to the initial course of therapy (Andersen and Mouridsen, 1988). Given the definition of the source population and follow-up time, all cases of recurrence occurred between 1 and 10 years after the primary breast cancer diagnosis.

Prescription status Prescription medications are coded by the Anatomical Therapeutic Chemical (ATC) classification system (WHO Collaborating Centre for Drug Statistics Methodology, 2007). We defined SSRI antidepressants as all those classified in group N06AB by the ATC. These are the SSRI drugs: zimeldine (N06AB02), fluoxetine (N06AB03), citalopram (N06AB04), paroxetine (N06AB05), sertraline (N06AB06), alaproclate (N06AB07), fluvoxamine (N06AB08), etoperidone (N06AB09), and escitalopram (N06AB10). We defined citalopram exposure as any prescription for citalopram (N06AB04) or its S-stereoisomer escitalopram (N06AB10).

We classified cases and controls as those with no record of a citalopram prescription during their follow-up time (never citalopram) and those with any record of prescription for citalopram during their follow-up time (ever citalopram). We used a similar procedure to classify cases and controls as ever or never users of another SSRI or of another prescription medication that is a CYP2D6 inhibitor or substrate, aside from those indicated to treat breast cancer recurrence or its effects. See the Supplementary online material for a complete list of these medications and the frequency of their use in the study population.

For group I women who ever had a citalopram prescription, we calculated the percentage of time on tamoxifen when they were simultaneously taking citalopram. We created categories of (a) intermittent citalopram use, defined as citalopram use overlapping tamoxifen use for more than 0% but less than 30% of the time on tamoxifen and (b) regular citalopram use, defined as citalopram use overlapping tamoxifen use for 30% or more of the time on tamoxifen. We chose 30% as the overlap boundary to allow sufficient sample size in the regular citalopram subgroup, while also investigating a substantial period of SSRI and tamoxifen comedication.

Covariates We defined the following set of covariates: (a) time period of breast cancer diagnosis (1985–1993, 1994–1996, and 1997–2001), (b) age at diagnosis (35–44 years, 45–54 years, 55–64 years, and 65–70 years), (c) menopausal status at diagnosis (premenopausal and postmenopausal), (d) county of residence at diagnosis (Aarhus, North Jutland, Viborg, and Ringkøbing), (e) UICC stage at diagnosis (stages I, II, and III), histological grade (grade I, II, III, and missing), surgery type (breast conserving surgery and mastectomy), and receipt of systemic adjuvant chemotherapy (yes and no), and (f) receipt of a prescription for another medication that is a CYP2D6 inhibitor or substrate, including other SSRI, while taking tamoxifen.

Analytic strategy

All analyses were conducted within strata of the two groups (oestrogen-receptor positive and treated with tamoxifen or oestrogen-receptor negative and not treated with tamoxifen). We computed the frequency and proportion of cases and controls within categories of assigned protocol of tamoxifen duration, of citalopram use, of use of other CYP2D6 inhibitors or substrates, and of the covariates. We calculated the number of cases and controls ever receiving citalopram, the number of total prescriptions for citalopram summed over all cases or controls, and the range of the number of prescriptions for citalopram received by each individual case or control.

We estimated the rate ratio associating citalopram prescription with breast cancer recurrence as the odds ratio (OR) in a conditional logistic regression including only citalopram use as the exposure variable and conditioned on the matched factors. By design, this ratio adjusts for confounding by the matched factors (Greenland, 2008). We examined whether the effect of citalopram use was modified by duration of tamoxifen therapy in a stratified analysis. Finally, we adjusted for residual confounding by the covariates that were not included in the matching by including them as independent variables in the conditional logistic regression. We retained in the final model any covariate that affected the log OR from the conditional logistic regression model associating citalopram use with breast cancer recurrence rate by more than 10% (Greenland, 1989). All estimates are accompanied by a 95% confidence interval (CI) calculated by the profile likelihood method. All analyses were performed using SAS version 9.

RESULTS

Table 1 shows the frequency and proportion of cases and controls, within strata of group, in the categories of the covariates. About two-thirds of cases and controls in both groups were diagnosed with primary breast cancer during the period 1997–2001, and the majority was resident in Aarhus or North Jutland counties, because the prescription registries began first in these two counties. A large majority had mastectomy as their primary surgical intervention, which is consistent with the clinical practice pattern previously reported in this region during this time period (Ahern *et al*, 2008). Group I women (positive oestrogen-receptor expression and treated with tamoxifen) were more likely to be post-menopausal (87%) than were group II women (66%; negative oestrogen-receptor expression and not treated with tamoxifen). Group I women were also less likely to receive systemic adjuvant chemotherapy (11 and 13% of cases and controls, respectively) than were group II women (80 and 70% of cases and controls, respectively); reflecting the preference for hormonal therapy over systemic adjuvant chemotherapy in women whose tumours expressed the oestrogen receptor. Between 3 and 11% of cases and controls ever used citalopram while taking tamoxifen (group I) or during their follow-up period (group II).

Table 2 depicts the pattern of SSRI prescriptions received by cases and controls. In both groups, SSRI prescriptions were primarily written for citalopram or its S-stereoisomer, escitalopram. For example, 17 of 23 group I cases (74%) ever prescribed an SSRI had at least one prescription for citalopram, accounting for 86% of the total number of prescriptions. Similarly, 22 of 30 group I controls (73%) ever prescribed an SSRI had at least one prescription for citalopram, accounting for 64% of their prescriptions. Sertraline accounted for the majority of the remaining prescriptions (11% of the total for cases and 23% for controls).

Group I women who ever used citalopram while taking tamoxifen did not have a higher rate of breast cancer recurrence than women who never used citalopram while taking tamoxifen (Table 3; OR = 0.79, 95% CI = 0.40, 1.6). This OR was not substantially modified by duration of tamoxifen therapy

Table 1 Frequency and proportion of cases of breast cancer recurrence and matched controls within group strata (I) expressing the oestrogen receptor and receiving at least 1 year of tamoxifen therapy (ERP+/TAM+), or (II) not expressing the oestrogen receptor, never receiving tamoxifen therapy, and surviving at least 1 year after diagnosis (ERP−/TAM−)

	Group I: ERP+/ TAM+ (*n* (%))		**Group II: ERP−/ TAM− (*n* (%))**	
	Cases	**Controls**	**Cases**	**Controls**
Citalopram prescription				
Ever	17 (9)	21 (11)	3 (3)	5 (6)
Never	167 (91)	163 (89)	84 (97)	82 (94)
Other CYP2D6 inhibitors, including other SSRI				
Ever	48 (26)	51 (28)	25 (29)	17 (20)
Never	136 (74)	133 (72)	62 (71)	70 (80)
Diagnosis year[a]				
1985–1993	33 (18)	34 (18)	13 (15)	11 (13)
1994–1996	32 (17)	29 (16)	17 (20)	18 (21)
1997–2001	119 (65)	121 (66)	57 (66)	58 (67)
Age at diagnosis				
35–44	13 (7)	11 (6)	15 (17)	12 (14)
45–55	38 (21)	34 (18)	37 (43)	29 (33)
55–65	91 (49)	93 (51)	26 (30)	29 (33)
65–70	42 (23)	46 (25)	9 (10)	17 (20)
Menopausal status at diagnosis[a]				
Premenopausal	24 (13)	24 (13)	30 (34)	30 (34)
Postmenopausal	160 (87)	160 (87)	57 (66)	57 (66)
County of residence at diagnosis[a]				
Aarhus	70 (38)	70 (38)	37 (43)	37 (43)
North Jutland	88 (48)	88 (48)	37 (43)	37 (43)
Viborg	15 (8)	15 (8)	9 (10)	9 (10)
Ringkøbing	11 (6)	11 (6)	4 (5)	4 (5)
UICC tumour stage at diagnosis[a]				
Stage I	7 (4)	7 (4)	4 (5)	4 (5)
Stage II	79 (43)	79 (43)	41 (47)	41 (47)
Stage III	98 (53)	98 (53)	42 (48)	42 (48)
Histological grade				
Grade I	31 (17)	33 (18)	4 (5)	17 (20)
Grade II	73 (40)	87 (47)	29 (33)	2 (2)
Grade III	44 (24)	24 (13)	38 (44)	22 (25)
Missing	36 (20)	40 (22)	16 (18)	46 (53)
Surgery type				
Breast conserving surgery	22 (12)	22 (12)	9 (10)	4 (5)
Mastectomy	162 (88)	162 (88)	78 (90)	83 (95)
Radiation therapy				
Yes	86 (47)	79 (43)	43 (49)	36 (41)
No	98 (53)	105 (57)	44 (51)	51 (59)
Tamoxifen protocol				
1 year	57 (31)	57 (31)	Not applicable	Not applicable
2 years	10 (5.4)	10 (5.4)		
5 years	117 (64)	117 (64)		
Systemic adjuvant chemotherapy				
Yes	21 (11)	24 (13)	70 (80)	61 (70)
No	163 (89)	160 (87)	17 (20)	26 (30)

[a]Variable included in risk set sampling to match controls to cases.

Table 2 Number of cases and controls receiving any prescription for each SSRI, and total number of prescriptions for each SSRI within group strata (I) expressing the oestrogen receptor and receiving at least 1 year of tamoxifen therapy (ERP+/TAM+), or (II) not expressing the oestrogen receptor, never receiving tamoxifen therapy, and surviving at least 1 year after diagnosis (ERP−/TAM−)

	Group I: ERP+/TAM+ *n* (no. of prescriptions) [range of no. per person]		**Group II: ERP−/TAM− *n* (no. of prescriptions) [range of no. per person]**	
SSRI name (ATC code)	**Cases**	**Controls**	**Cases**	**Control**
Zimeldine (N06AB02)	0	0	0	0
Fluoxetine (N06AB03)	1 (4) [4–4]	4 (24) [1–13]	2 (12) [1–11]	0
Citalopram (N06AB04)[a]	16 (251) [1–53]	21 (123) [1–24]	3 (4) [1–2]	5 (64) [1–43]
Paroxetine (N06AB05)	2 (6) [1–5]	1 (1) [1–1]	1 (2) [2–2]	3 (20) [5–9]
Sertraline (N06AB06)	3 (32) [3–24]	7 (45) [1–15]	1 (2) [2–2]	1 (12) [12–12]
Alaproclate (N06AB07)	0	0	0	0
Fluvoxamine (N06AB08)	0	0	0	0
Etoperidone (N06AB09)	0	0	0	0
Escitalopram (N06AB10)[a]	1 (2) [2–2]	1 (3) [3–3]	0	0

[a]In the analysis, we defined citalopram exposure as any prescription for citalopram (N06AB04) or its S-stereoisomer escitalopram (N06AB10).

Table 3 Association between SSRI prescription and breast cancer recurrence within strata of (a) Group I, women with tumours that expressed the oestrogen receptor and who received at least 1 year of tamoxifen therapy (ERP+/TAM+) or (b) Group II, women with tumours that did not express the oestrogen receptor, who never received tamoxifen therapy, and who survived at least 1 year after diagnosis (ERP−/TAM−)

Citalopram prescription	**Cases/controls**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)[a]**
(a) Group I: ERP+/TAM+			
Never user	167/163	1.0 (reference)	1.0 (reference)
Ever user	17/21	0.79 (0.40, 1.6)	0.85 (0.42, 1.7)
Intermittent use	10/14	0.69 (0.30,1.6)	0.72 (0.30, 1.7)
Regular use	7/7	0.97 (0.34, 2.8)	1.1 (0.37, 3.3)
(b) Group II: ERP−/TAM−			
Never user	84/82	1.0 (reference)	1.0 (reference)
Ever user	3/5	0.60 (0.14, 2.5)	0.78 (0.17, 3.6)

[a]Adjusted for age category and other CYP2D6-inhibiting medications (see the Supplementary online material for a complete list of these medications and the frequency of their use in the study population).

($P = 0.23$ for test of homogeneity; data not shown). The approximately null effect persisted with adjustment for age category and ever/never use of another CYP2D6 inhibitor or SSRI (OR = 0.85, 95% CI 0.42, 1.7). The effects were likewise approximately null within cumulative citalopram prescription categories (intermittent use OR = 0.72, 95% CI 0.30, 1.7; regular use OR = 1.1, 95% CI 0.37, 3.3). Citalopram use also had no substantial effect on recurrence in group II women (adjusted OR = 0.78, 95% CI 0.17, 3.6), suggesting that citalopram does not directly affect the risk of breast cancer recurrence.

DISCUSSION

The results of this study do not support the hypothesis that citalopram, taken concurrently with tamoxifen, reduces tamoxifen's protective effect against breast cancer recurrence in early-stage patients whose tumour cells express the oestrogen receptor.

Our results extend the findings from an earlier study of 28 stage II and III breast cancer patients with recurrence and their matched controls at a single United States oncology centre, which also reported no substantial modification of tamoxifen effectiveness by concomitant use of SSRI inhibitors of CYP2D6 (Lehmann *et al*, 2004). These results may seem at odds with the strong biological rationale and *in vivo* evidence that support the hypothesis that CYP2D6 inhibition would reduce tamoxifen's prevention of breast cancer recurrence. It is possible, however, that SSRI medications could reduce the plasma concentration of tamoxifen's secondary metabolites without reducing its anti-tumorigenicity (Ponzone *et al*, 2004; Ratliff *et al*, 2004; Stearns *et al*, 2004). Tamoxifen doses as much as 20-fold lower than the typical US dose of 20 mg day^{-1} affect biomarkers of cardiovascular, bone, and tumour end points (Decensi *et al*, 1998, 2003), so the approximately three-fold reduction in the plasma concentration of tamoxifen's secondary metabolites associated with concomitant receipt of the SSRI paroxetine (Jin *et al*, 2005) may have little consequence.

The key mechanistic question may be whether reduced concentrations of active tamoxifen metabolites result in substantially reduced occupancy of the oestrogen receptor. Dowsett and Haynes (2003) estimated that, in postmenopausal women on a daily dose of 20 mg tamoxifen, tamoxifen and its metabolites occupy 9994 of 10 000 oestrogen receptors. Replicating their calculation using the plasma concentrations of tamoxifen and its metabolites in women with no *CYP2D6* variant allele (Jin *et al*, 2005), tamoxifen and its metabolites would occupy 9999 of 10 000 receptors in women not taking any SSRI and 9997 of 10 000 receptors in women taking the strong *CYP2D6*-inhibiting SSRI paroxetine. Steady-state concentrations of tamoxifen and its metabolites may be sufficient to manifest fully tamoxifen's antitumorigenic effect in postmenopausal women regardless of whether *CYP2D6* inhibition reduces the concentration of some tamoxifen metabolites.

Nonetheless, our results should be considered with the following limitations in mind. First, the majority of SSRI prescriptions in our study were for citalopram or its S-stereoisomer, both originally manufactured by Lundbeck, a company headquartered in Denmark. Citalopram is a modest inhibitor of *CYP2D6* compared with some other SSRI medications (Jeppesen *et al*, 1996). These more potent inhibitors may reduce tamoxifen's protection against breast cancer recurrence, but their interaction with tamoxifen would not have been well measured by this study.

Second, we have not collected genotype data to characterize functional *CYP2D6* variants (Hayhurst *et al*, 2001) that affect the metabolism of tamoxifen (Jin *et al*, 2005). The combination of genotype and receipt of *CYP2D6*-inhibiting medications has been related to tamoxifen effectiveness in a previous study (Goetz *et al*, 2007). We do not, however, expect ever-receipt of citalopram while taking tamoxifen to be related to *CYP2D6* genotype, as this genotype would be unknown to the patient and provider at the first citalopram prescription. This study's results therefore pertain to the usual clinical setting. In addition, *CYP2D6* genotype is unlikely to cause citalopram prescription, or to share a common causal ancestor, so *CYP2D6* genotype does not satisfy the requisite causal structure of a confounder (Greenland *et al*, 1999). It may be possible that *CYP2D6* genotype is related to adherence to citalopram prescription or to long-term maintenance of the prescription, resulting from differences in the occurrence of adverse drug reactions in women with the different alleles. Such a relation could confound the association between breast cancer recurrence and duration of citalopram prescription while taking tamoxifen. Some non-randomized studies suggest such a relation between genotype and SSRI adherence (Rau *et al*, 2004; Zourková *et al*, 2007), whereas others suggest no such relation (Stedman *et al*, 2002; Gerstenberg *et al*, 2003; Roberts *et al*, 2004; Hedenmalm *et al*, 2006; Sugai *et al*, 2006; Suzuki *et al*, 2006). In the only randomized trial, *CYP2D6* genotype was not related to either the occurrence of adverse events or to adherence to paroxetine prescription (Murphy *et al*, 2003). Paroxetine is the most potent *CYP2D6* inhibitor of tamoxifen metabolism among the SSRI class (Jin *et al*, 2005). If *CYP2D6* genotype does not affect receipt or adherence to SSRI prescription, then it cannot confound the association we have reported.

Last, we do not know the indications for which citalopram was prescribed to the study participants, although ordinarily it would be prescribed primarily to treat depression. SSRI may also be prescribed to treat hot flushes (Stearns, 2006), but such prescriptions are rare in Danish breast cancer patients.

Weighing against these limitations are the strengths of the data quality. This study relied upon the Danish Breast Cancer Cooperative Group's registry of breast cancer patients, which provides clinical trial quality data in a population-wide setting in the four Northern Danish Counties. For example, the positive predictive value of breast cancer recurrence recorded by the DBCG equaled 99.4% in a validation study (Hansen *et al*, 1997), showing that there are few false-positive recurrences registered in the DBCG. In addition, of 1888 local and distant recurrences identified by medical record review among 4455 breast cancer patients assigned to a DBCG protocol, 1813 (96%) were correctly registered as recurrences in the DBCG database, 74 (3.9%) were identified as breast cancer deaths, and only 1 (0.05%) was not identified as either a recurrence or breast cancer death.

The prescription databases are generated by a computerised pharmacy accounting system that sends data to the Danish National Health Service, which refunds part of the costs associated with prescribed drugs. Given the direct connection between receipt of prescription medications and the pharmacy accounting system of the Danish National Health Service, we expect the prescription records to have excellent validity. The prescriptions from the four counties are merged into a research database at Aarhus University. In Denmark, antidepressants are available only at pharmacies and the patient must have a prescription from a medical doctor. Therefore, the county prescription databases are expected to have high sensitivity and specificity for ascertainment of citalopram prescriptions in the source population. Furthermore, because the prescription records antecede the date of breast cancer recurrence, they are a prospective data source presumably immune to differential classification bias (Rothman *et al*, 2008).

Despite these advantages, the study yielded only 17 cases of breast cancer recurrence among tamoxifen-treated women who had used citalopram while taking tamoxifen. The study was designed with 80% power to detect an OR of 1.6, and ultimately had 90% power to detect an OR of 2.3.

The results presented herein are, nonetheless, important and timely. A United States Food and Drug Administration advisory committee recently recommended relabelling tamoxifen with information on gene–drug and drug–drug interactions mediated by *CYP2D6* (American Cancer Society, 2007). Furthermore, the current practice guidelines of the United States National Comprehensive Cancer Network note that some SSRI reduce the formation of active tamoxifen metabolites, that citalopram and venlaflaxine appear to have minimal impact on tamoxifen metabolism, and that 'the clinical impact of these observations is not known' (National Comprehensive Cancer Network, 2008). Breast cancer patients taking tamoxifen and their physicians may therefore be concerned about SSRI comedication, even when antidepressants are strongly indicated. Our results suggest that citalopram prescription does not reduce tamoxifen's prevention of breast cancer recurrence.

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CANCER

Trends in breast-conserving surgery in Denmark, 1982–2002

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Abstract Using hospital discharge data from the counties in Northern Denmark and the Danish Cancer Registry, we examined the trend in the prevalence of breast-conserving surgery (BCS) to treat primary breast cancer from 1982 through 2002, with an emphasis on publications that may have influenced surgical practice in Denmark. Overall, the prevalence of BCS increased from less than 1% of breast cancer operations in 1982 to approximately 25% by 2002. The rise in prevalence was most pronounced for the treatment of young women and women with early-stage breast cancer. Of three pivotal clinical trials, the most significant trigger of the upward trend appeared to be a study conducted by the Danish Breast Cancer Cooperative Group, published in 1988. After 1988, there was a steep rise in the prevalence of BCS. By 2002, BCS prevalence appeared to reach a threshold at 25% of breast cancer operations, seemingly defined by the proportion of new breast cancer cases who are good candidates for BCS.

Keywords Epidemiology · Breast Neoplasms · Mastectomy · Segmental

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Abbreviations

BCS	Breast-conserving surgery
BCS-RT	Breast-conserving surgery with radiotherapy
CPR	Civil personal registration number
DBCG	Danish Breast Cancer Cooperative Group
DCR	Danish cancer registry
HDR	Hospital discharge registry
ICD	International Classification of Diseases
NSABP	National Surgical Adjuvant Breast and Bowel Project
SEER	Surveillance, epidemiology and end results
UICC	International Union Against Cancer

Introduction

Before the 1980s, surgical treatment of breast cancer was almost entirely accomplished by Halsted's radical mastectomy procedure, first published in 1898, or modifications thereof [1]. Despite pervasive use of radical mastectomy to treat all types of breast cancer, sporadic case reports of breast-conserving surgery (BCS) appeared in the literature throughout the 1970s [1–3]. The motivation to develop this procedure apparently stemmed from increasing detection of breast cancer cases at early stages, for which the traditional radical mastectomy seemed an overtreatment [1]. The breast-conserving procedure was characterized by local excision of the tumour with follow-on radiotherapy to ablate occult tumour foci remaining in the breast. The first clinical trial comparing BCS and radiotherapy to mastectomy was published in 1972, and showed that BCS, compared to mastectomy, resulted in a higher incidence of local and distant recurrences as well as significantly reduced 10-year overall survival in

node-positive patients (Manchester stage 2) [4]. This result was later attributed to a naïveté regarding the importance of tumour-free tissue margins and an insufficient radiation dose (32 Gy) given in the BCS treatment regimen [1]. Preliminary results from subsequent trials comparing BCS coupled with radiotherapy (BCS-RT) to mastectomy were published in the 1980s and early 1990s, [5–8] with long-term updates appearing thereafter [9–13]. The combined evidence of these studies overwhelmingly supported equivalency of BCS-RT to mastectomy in patients with early stage breast cancer (UICC stage I or II) with respect to both disease-free and overall survival [5–13]. In addition, cosmetic and adverse effect outcomes were more favorable, on average, for patients undergoing BCS [5].

The aim of this descriptive study is to examine the trend in BCS prevalence among all primary treatment operations for patients with non-metastatic breast cancer in the northern part of Denmark from 1982 through 2002, with reference to important publication events that may have influenced the adoption of BCS over mastectomy during that time period.

Patients and methods

Landmark clinical trial ascertainment

A literature search was conducted to identify major clinical trial results comparing BCS to mastectomy, which were likely to influence surgical practice in Denmark. Three randomized clinical trials were deemed most influential to the Danish breast surgical community over the time period: a trial conducted at the Milan Cancer Institute and published in 1981 [7], a similar trial published in 1985 by the U.S. National Surgical Adjuvant Breast and Bowel Project (NSABP Protocol B-06) [6], and a trial conducted by the Danish Breast Cancer Cooperative Group (DBCG-82TM), published in 1988 [5]. Preliminary reports were issued from these trials in the 1980s, with periodic updates published throughout the remainder of the study period.

Breast cancer surgical data collection

We identified surgical procedures for women (either mastectomy or BCS) related to a diagnosis of breast cancer by linking the hospital discharge registries of three Danish counties (North Jutland, Viborg, and Aarhus; population 1.4 million) to the Danish cancer registry (DCR). A hospital discharge registry has been in operation in each Danish county since 1977 (in Viborg County since 1972) and records dates of admission and discharge, surgical procedure(s) performed, and up to 20 discharge diagnoses immediately after the discharge of the patient. Data from the hospital discharge registries from the three counties have been merged into a research database at Aarhus University, Denmark and linked to data from the DCR. Patients are identified in the databases by their civil personal registration (CPR) numbers, a unique number issued to all Danish residents at birth or emigration that encodes gender and birth date. The CPR number is used by all Danish registries and facilitated linkage of the hospital discharge registry data to the DCR for this study.

Using the HDR we identified the first operation sequence related to a breast cancer diagnosis for each woman in the register. An operation sequence was considered to be related to a breast cancer diagnosis if a diagnosis was given at the time of discharge, or if a diagnosis was registered in a separate admission record with a discharge date within 90 days of the surgical date. Breast cancer diagnoses and surgical procedures were classified in the HDR using ICD-8 (until 1993) and ICD-10 (from 1994 forward) codes. Complete data were available for the three counties from January 1, 1982 to December 31, 2002. No organized screening for breast cancer by mammography occurred in the study area during this period.

About 15,502 records meeting the inclusion criteria were identified, representing 14,487 women with a total of 15,605 operations. For women who received more than one breast cancer operation within a 60 day period (for instance, BCS followed by mastectomy), we defined the most recent procedure as the surgical treatment type. We excluded records for women with no registration in the DCR (N = 1,087), women whose treatment course did not fall entirely within the date ranges examined (N = 1,367), women whose first DCR registration was for bilateral cancer or if two unilateral cancers were recorded on the same date (N = 610), women with duplicate DCR records for the same breast (N = 14), women for whom more than 6 months had elapsed between the first operation and appearance of the DCR record (N = 236), and women whose surgical sequences were inconsistent (for instance, first operation being a mastectomy followed by a record of BCS), (N = 20). We also excluded records for women with metastatic disease, since the choice of operation type for these patients depends upon a different set of factors and clinical expectations than the choice for women with local or regional disease. After applying these exclusion criteria, 10,775 women remained in the analysis.

We obtained data on summary disease stage (classified as local, regional or metastatic) from the DCR. Summary staging is commonly employed by cancer registries and provides a broader categorization of disease characteristics than the clinical TNM staging systems. This feature of summary staging allowed us to evaluate trends within

levels of stage without using the more finely divided clinical staging categories. In certain cases, a summary stage cannot be reliably assigned either due to incomplete diagnostic data or contradictory reports. Out of the 10,775 women in the analysis, 480 (4.5%) were classified as unknown stage.

Data analysis

The prevalence of BCS among all breast cancer surgeries was computed for each year during the study period, based on the date of the definitive surgical procedure. Patient age at the time of surgery was categorized into approximate quartiles (20–49, 50–59, 60–69 and 70–100 years) based on the univariate distribution in the entire sample. One subject in the data set had a recorded age of 8 years and was excluded from the analysis.

Smoothed plots of BCS prevalance were generated by averaging the monthly proportions across a 5-month window, and advancing this window 1 month at a time. Proportions at the center of the window were weighted more heavily than proportions at the window edges. These smoothed proportions were divided into four time periods defined by the intervals between (A) publication of the Milan and NSABP studies, (B) publication of the NSABP and DBCG studies, (C) publication of the DBCG study and the first report of fraud within the NSABP trial [10], and (D) the report of fraud and the end of the study period. The trend within each time interval was fit with a cubic spline function generated by maximizing the binomial likelihood of the observations within the interval [14]. These plots were generated for the crude trend as well as within strata of stage (local and regional) and strata of age (20–49, 50–69 and 70–100 years). Markers indicating the publication year of major clinical trial results comparing BCS-RT to mastectomy were included on all of the plots. Differences in the distribution of age and stage categories between surgical groups were assessed by Pearson's chi-squared tests. Two-sided *P*-values testing the null hypothesis are reported. All analyses were performed with the SAS version 9.1 (SAS Institute, Cary, North Carolina, USA).

Results

Landmark clinical trials

The first of three definitive clinical trials comparing BCS to mastectomy was conducted at the National Cancer Institute in Milan, Italy. It randomized women with stage I breast cancer (tumour diameter $\leq$ 2 cm) either to Halsted radical mastectomy or to quadrantectomy with combination radiotherapy [7]. After 7 years of follow-up, the trial data showed similar disease-free and overall survival rates for the two treatment groups, with fewer post-operative complications reported in the BCS group [7].

The second trial, performed by the U.S. National Surgical Adjuvant Breast Project, compared total mastectomy to segmental mastectomy, with our without radiation therapy. The segmental mastectomy procedure was considerably more conservative than the quadrantectomy used in the Milan study; it stipulated tissue resection only to the extent that excised specimen margins were free of tumour. The NSABP trial reached the same main conclusion as the Milan trial; mastectomy and BCS-RT were equivalent with respect to disease-free and overall survival. More importantly, the trial results provided strong evidence that radiotherapy is of great additional benefit to reduce recurrence risk among those subjects undergoing BCS, regardless of their age, nodal status and tumour size.

The third trial was conducted by the Danish Breast Cancer Cooperative Group (DBCG-82TM) [5], when the surgical standard for breast cancer treatment in Denmark solely consisted of total mastectomy with dissection of lower axillary lymph nodes. Patients either received standard mastectomy or a BCS procedure similar to that employed in the NSABP trial. All subjects who underwent BCS received postoperative radiotherapy. The trial's preliminary results, published in 1988 after 6 years of follow-up, indicated equivalence of BCS-RT to mastectomy with respect to recurrence-free survival. The results also indicated that approximately 25% of newly diagnosed breast cancer cases at the participating clinics were candidates for BCS.

Trend in breast-conserving surgery

Of the 10,775 women in the data set, 1,461 (13.6%) underwent BCS. Distributions of age and disease stage between the mastectomy and BCS groups are shown in Table 1. The median age of the women who received BCS was 8 years younger than that of the women who received mastectomy (53.8 and 61.9 years, respectively). As would be expected, women who received BCS were more likely to have less-advanced disease than women who received mastectomy.

Figure 1 shows the overall upward trend in the proportion of breast-conserving procedures in the three Danish counties between 1982 and 2002. These crude data are derived from 10,772 records, and the total number of breast cancer operations performed each year ranged from 334 to 759. There are two small increases in the prevalence of BCS, which are seen in the data points but are not reflected in the smoothed curve, following publication of both the

Table 1 Characteristics of surgical treatment groups, *N* (row %)

	Mastectomy *N* = 9,313	Breast-conserving *N* = 1,462	*P*-value[a]
Age (years)			
20–49	2,174 (80.0)	544 (20.0)	<0.0001
50–59	2,086 (82.7)	438 (17.4)	
60–69	2,197 (88.8)	276 (11.2)	
70–100	2,856 (93.3)	204 (6.7)	
Summary disease stage			
Local	4,738 (83.4)	945 (16.6)	<0.0001
Regional	4,148 (89.9)	465 (10.1)	
Unknown	428 (89.2)	52 (10.8)	

[a] Two-sided *P*-values from Pearson's chi-square test, $\alpha = 0.05$

Milan and NSABP trials, with a more considerable upward jump following publication of the DBCG trial's preliminary results. Over the study period, overall BCS prevalence rose from 0.9% in 1982 to 25.2% in 2002.

The stage-stratified trend plot shown in Fig. 2 shows a steeper rise in prevalence of BCS for the treatment of local stage disease compared to the trend for regional disease. Both curves start at 1982 with approximately 1% prevalence; they begin to diverge following publication of the preliminary NSABP results in 1985. In 2002, BCS prevalence was 31.7% and 18.7% in the local and regional strata, respectively.

The most striking difference in BCS trend was seen across age categories, as shown in Fig. 3. A pronounced jump in the use of breast-conserving procedures for subjects aged 20–49 was seen immediately following publication of preliminary results from DBCG-82TM. A similar though somewhat attenuated trend was seen for women aged 50–69, whereas the trend for women aged 70–100 remained relatively flat. In 2002, BCS prevalence was 27.9% among women aged 20–49, 28.4% among women aged 50–69, and 16.7% among women aged 70–100. When the youngest age stratum was restricted to women with local disease, the proportion receiving BCS in 2002 increased to 32.5% (data not shown). Since the presence of axillary metastases is strongly correlated with tumour size, and because tumour size is a stronger determinant than axillary involvement in the choice of BCS, this finding likely reflects an effect of smaller tumour size (not an effect of axillary involvement) on the use of BCS.

Discussion

To achieve adequate margins and an acceptable cosmetic result in BCS, the relative size of the tumour to the breast and the location of the tumour in the breast are important factors. The smaller the tumour, the greater the possibility for BCS. In the counties included in the study, no organized screening by mammography occurred during the study period and it is unlikely that the observed increase in BCS prevalence was caused by an increase in patient-requested mammography. Rather, it is likely the result of an increasing acceptance of the procedure among surgeons and patients. It is also possible that BCS may have been widely introduced in Denmark earlier, had it not been that the majority of Danish surgeons were awaiting the results of the DBCG-82TM trial, in which many of them participated.

In 1991, 2 years after publication of 8-year follow up results of the NSABP trial, the NSABP verified that falsified data had been reported by St. Luc Hospital in

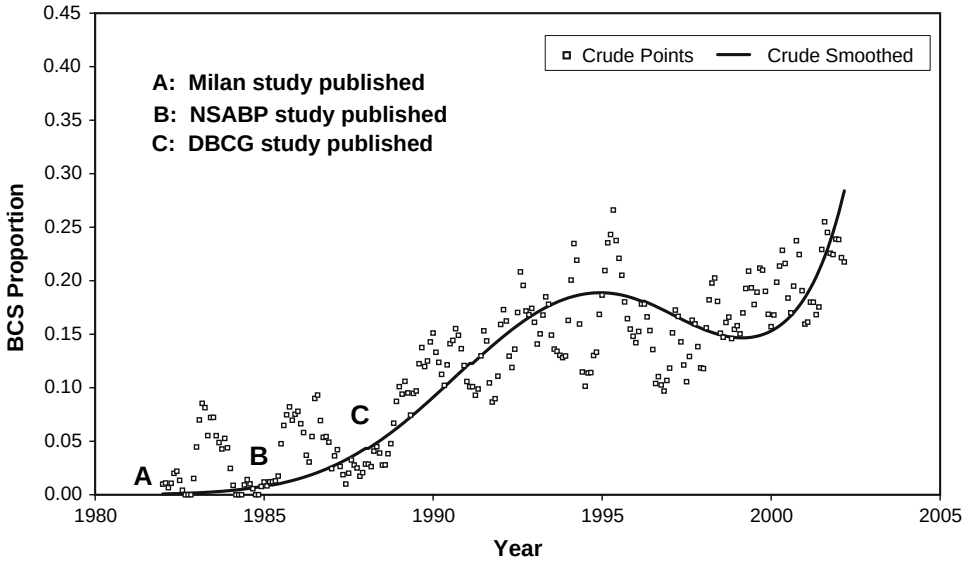

Fig. 1 Overall trend in breast-conserving surgery prevalence in North Jutland, Viborg and Aarhus counties, Denmark, 1982–2002

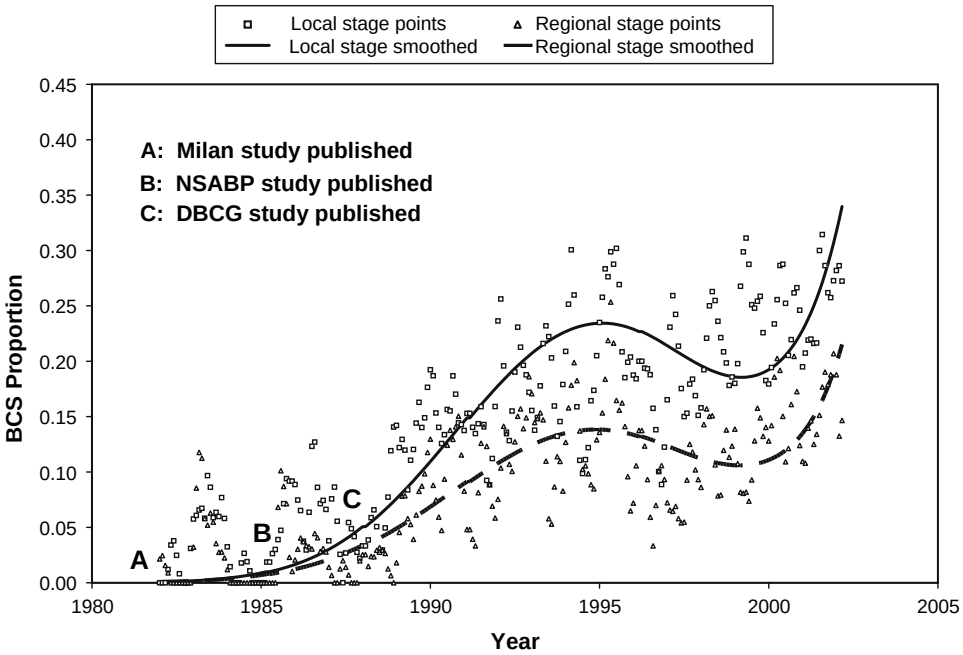

Fig. 2 Trend in breast-conserving surgery prevalence for local and regional disease stages, 1982–2002

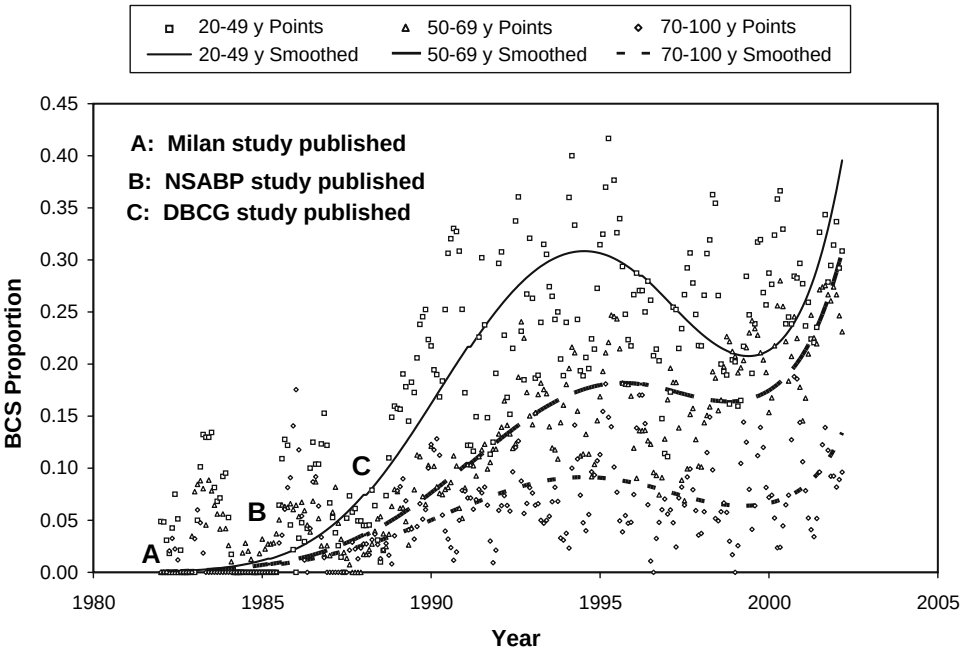

Fig. 3 Trend in breast-conserving surgery in women aged 20–49, 50–69 and 70–100 years

Montreal, a participating site in the B-06 study. Saint Luc Hospital had enrolled a total of 354 research subjects, six of whom had false biopsy dates reported to the NSABP headquarters. The report of fraud received considerable media attention and was followed by an extensive audit of the NSABP Protocol B-06 data in 1994, which uncovered no further corruption of data. The exonerated investigators re-analyzed the trial results with and without the fraudulent subjects included and published their results along with 12-year follow up data in 1995; conclusions from both analyses did not differ, again confirming the equivalence of BCS-RT to mastectomy [10]. Whether these events impacted on the dip in BCS prevalence around 1995 is not testable but is worth noting, especially since the trend resumed its climb following publication of the confirmed results.

The authors of the first report from DBCG-82TM noted that approximately 25% of the incident breast cancer cases presenting to the participating clinics were eligible for BCS [5]. This figure matches the overall prevalence of BCS in the three Danish counties in 2002. Twenty-five percent may be a "prevalence ceiling" for BCS, restricted by the

proportion of eligible cases in the population, and may have been reached by that year. If this conclusion is correct, then no further rise in the prevalence of BCS can be expected unless more pervasive mammographic screening programs for asymptomatic women are successfully initiated in Denmark, whereby cases will be detected in younger women and at earlier disease stages. Recent legislation to promote such screening practices by 2008 may lead to a future increase in the prevalence of BCS. In Sweden, where nationwide coverage for screening mammography began in 1991 [15], the overall prevalence of BCS increased from 7% in 1980 to 18% in 1985, and was 51% in 1995, 4 years after the initiation of screening mammography [16]. In the United States, where surveillance mammography became widespread in the 1980s, the prevalence of BCS rose from approximately 30% in 1988 to approximately 60% by 1998 [17].

Our study sample was the combined populations of North Jutland, Viborg and Aarhus counties (1.4 million people; approximately 26% of the total population of Denmark). While a larger sample would have yielded more precise prevalence estimates, the actual estimates and their trend over time in the whole of Denmark should be accurately depicted by the three chosen counties. The Danish Breast Cancer Cooperative Group was established in 1976 to ensure optimal diagnosis and treatment of operable primary breast cancer on a nationwide basis [18]. Because the DBCG directs breast cancer treatment protocols on a national level, there is no reason to suspect substantially different treatment patterns by geographic region.

In conclusion, the prevalence of BCS increased from approximately 1 to 25% of all breast cancer operations in Viborg, North Jutland and Aarhus counties in Denmark during the period 1982–2002. The prevalence rose most considerably following publication of initial results from a Danish clinical trial comparing BCS to mastectomy, which showed equivalence of the two procedures with respect to recurrence-free survival among women with invasive breast carcinoma. By 2002, the prevalence appeared to reach a plateau, perhaps defined by the proportion of breast cancer cases in the Danish population who are good candidates for BCS.

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