FULL PAPER

BJC

British Journal of Cancer (2016) 115, 382–390 | doi: 10.1038/bjc.2016.174

Keywords: breast cancer; smoking; endocrine treatment; aromatase inhibitor; tamoxifen; prognosis

Impacts of smoking on endocrine treatment response in a prospective breast cancer cohort

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Background: The association between smoking and breast cancer prognosis remains unclear. The purpose of this study was to investigate whether preoperative smoking was associated with prognosis in different treatment groups.

Methods: This population-based cohort consisted of 1065 breast cancer patients without preoperative treatment included between 2002 and 2012 in Lund, Sweden. Smoking status was examined in relation to patient and tumour characteristics, and prognosis in different treatment groups.

Results: At the preoperative visit, 21.0% smoked. Median follow-up time was 5.1 years. Overall, in the 1016 patients included in the survival analyses, there was no significant association between smoking and risk of breast cancer events (adjusted hazard ratio (adjHR): 1.45; 95% confidence interval (CI): 0.95–2.20). For the 309 aromatase inhibitor (AI)-treated patients ⩾50 years with oestrogen receptor-positive (ER+) tumours, smoking was associated with risk of breast cancer events (adjHR: 2.97; 95% CI: 1.44–6.13), distant metastasis (adjHR: 4.19; 95% CI: 1.81–9.72), and death (adjHR: 3.52; 95% CI: 1.59–7.81). Smoking was not associated with breast cancer events or distant metastasis in other treatment groups.

Conclusions: Preoperative smoking was only associated with an increased risk for breast cancer events and distant metastasis in AI-treated patients. If confirmed, smoking status should be taken into consideration when selecting an endocrine therapy.

Breast cancer is the most common cancer among women worldwide (Ferlay *et al*, 2014). Identification of modifiable lifestyle factors that may improve prognosis is of interest to women diagnosed with breast cancer. Several studies have investigated the association between smoking and prognosis in breast cancer patients. Smoking is associated with an overall increased mortality. However, the association between smoking and breast cancer-specific mortality or disease-free survival remains unclear. Some studies found no significant association (Holmes *et al*, 2007; Berube *et al*, 2014; Seibold *et al*, 2014), whereas others found an increased risk for recurrence or breast cancer-specific mortality (Manjer *et al*, 2000; Braithwaite *et al*, 2012; Bishop *et al*, 2014; Pierce *et al*, 2014; Nechuta *et al*, 2016; Passarelli *et al*, 2016). Moreover, this association was sometimes only reported in current smokers or in patients with more extensive smoking.

In 2013, ~11% of the female population in Sweden smoked cigarettes daily, and an additional 9% smoked occasionally (Public Health Agency of Sweden (Folkhälsomyndigheten, 2013). Cigarette smoke contains over 7000 chemicals, 69 of which are established carcinogens (United States Department of Health and Human Services, 2010). Smoking has anti-oestrogenic effects and decreases endogenous oestrogen (Baron, 1984). Constituents of cigarettes such as nicotine and other tobacco alkaloids inhibited oestrogen synthesis via the aromatase enzyme when tested *in vitro*

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Received 16 November 2015; revised 11 May 2016; accepted 16 May 2016; published online 9 June 2016

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(Barbieri *et al*, 1986; Kadohama *et al*, 1993). However, current smoking is also associated with increased levels of urinary prostaglandin E_2 metabolites (Kim *et al*, 2013). Prostaglandin E_2 is synthesised from arachidonic acid via the action of cyclooxygenase 2 and is a key mediator of inflammation (Park *et al*, 2006) that increases aromatase activation (Subbaramaiah *et al*, 2012). The net effect of smoking on aromatase activity in different women may vary.

Little is known about how smoking influences response to different types of breast cancer treatments. Smoking has been reported to impact the response to both radiation and chemotherapy, primarily in other cancers (An *et al*, 2012; Hoff *et al*, 2012; Trevino *et al*, 2012; Bishop *et al*, 2014; Guha *et al*, 2014). Endocrine therapy represents one of the most effective treatments for women with oestrogen receptor-positive (ER+) breast cancers. Two classes of agents are the selective ER modulators, for example, tamoxifen (TAM) and aromatase inhibitors (AIs) (Jordan, 2006; Dowsett *et al*, 2010). To our knowledge, no study has investigated a potential association between smoking and response to different types of endocrine therapy. We hypothesise that smoking may be associated with the response to endocrine treatment as smoking affects endogenous oestrogen levels via the aromatase enzyme, and that any association may differ between TAM and AIs because of their different mechanisms of action.

The aim of this study was to examine if the prognosis differed between smokers and non-smokers among patients who had received different types of breast cancer treatment, with a special focus on the endocrine treatment response.

MATERIALS AND METHODS

Patients of all ages diagnosed with a first breast cancer at the Skåne University Hospital in Lund, Sweden were included between October 2002 and June 2012 in an ongoing prospective cohort study of lifestyle factors and their association with prognosis and treatment response ($n=1116$). There were a total of 2170 female patients operated for breast cancer during the time period this cohort was compiled. Only patients with primary breast cancer and no other cancer during the past 10 years were included in the cohort. The total number also included patients with a secondary breast cancer as well as patients who had been diagnosed with other cancers within the past 10 years. The median age of all patients was 61 years. Oestrogen receptor status was available for 1928 patients, of whom 85.4% had ER+ tumours. Progesterone receptor (PgR) status was available for 1914 patients, of whom 70.1% had PgR+ tumours. For the present study, 51 patients who had received preoperative treatment were excluded, leaving 1065 patients (see Figure 1). The study was approved by the Lund University Ethics Committee (LU Dnr75-02, Dnr37-08, Dnr658-09, Dnr58-12, Dnr379-12, Dnr 277-15, Dnr458-15), and written informed consents were obtained from all participants.

Participating patients completed a three-page questionnaire at the preoperative visit. Follow-up questionnaires were completed 3–6 months, and 1, 2, 3, 5, 7, 9 and 11 years postoperatively. The questionnaire included questions regarding medication intake during the last week, reproductive history, and smoking and alcohol consumption. The follow-up questionnaires also provided information on adjuvant treatment. Anthropometric measures including height, weight, waist and hip circumferences, and breast volume were measured with plastic cups by trained research nurses, as described previously (Ringberg *et al*, 2006; Markkula *et al*, 2012a).

The patients were asked to define themselves as non-smokers, smokers, or occasional smokers. The approximate number of cigarettes consumed during the last week was obtained as an interval (0, 1–5, 6–10, 11–15, 16–20, 20+). Patients who considered themselves as either smokers or occasional smokers or who had reported to have smoked >0 cigarettes were defined as 'smokers'.

Tumours were analysed at the Department of Pathology at the Skåne University Hospital, Lund, Sweden. Information on tumour size, axillary lymph node involvement, histological grade, and ER and PgR status (positive if $>10\%$ nuclei were stained according to standard clinical practice in Sweden) was obtained from each patient's pathology report, as described previously (Bågeman *et al*, 2008; Jernström *et al*, 2009; Simonsson *et al*, 2014). The human epidermal growth factor receptor 2 (HER2) status was routinely analysed as of November 2005 in patients younger than 70 years of age with invasive tumours, as described previously (Markkula *et al*, 2014), and is thus missing for a substantial part of the tumours. Analyses of Ki-67 index were routinely performed as of March 2009 and are therefore not included.

Information regarding breast cancer events – defined as either local or regional recurrence, new breast cancer, or distant metastases – and date of death owing to any cause was collected from the patients' charts, pathology reports, regional tumour registry, and population registry. Information regarding treatment was obtained from the patients' charts as well as from the questionnaires. Only treatment before any breast cancer event was considered. The study was observational, and treatment was provided according to the standard of care at the Skåne University Hospital.

Statistical analysis. All statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) version 19.0 (IBM Corp., Armonk, NY, USA). Patient and tumour characteristics were analysed in relation to smoking status at the preoperative visit. Total breast volume for both breasts was calculated for those with no previous breast surgery (Ringberg *et al*, 2006; Markkula *et al*, 2012a). Tumour characteristics included invasive tumour size ($\leqslant 20$ mm, 21–50 mm, $\geqslant 51$ mm, muscle or skin involvement or $\geqslant 21$ mm, or muscle or skin involvement (yes/no)), pathological axillary lymph node involvement (0, 1–3, 4+, or axillary lymph node involvement (yes/no)), histological grade (I–III or grade III (yes/no)), hormone receptor status (ER+, PgR+), and HER2 status (amplified/not amplified). For analysis of categorical variables in relation to smoking status, Pearson's χ^2 test was used. If the expected number of patients in one or more categories was <5, Fisher's exact test was used. Continuous variables were analysed using the Mann–Whitney *U*-test for the univariable analyses. Variables that were not normally distributed were categorised for the multivariable analyses.

Response to given treatments, measured as risk of breast cancer events, was analysed in relation to preoperative smoking status. For these analysis, patients with carcinoma *in situ* ($n=39$), metastatic spread within 0.3 years from inclusion ($n=8$), or missing preoperative smoking status ($n=2$) were excluded. The follow-up time was calculated as the time from inclusion until a first breast cancer event, death from a non-breast cancer-related cause, or last follow-up for patients who were alive and event-free before 1 July 2014. Patients were censored at the time of a non-breast cancer-related death or last follow-up. Similarly, follow-up time until distant metastasis was calculated as the time from inclusion until a first distant metastasis, death from a non-breast cancer-related cause, or last follow-up for patients who were alive and distant metastasis-free before 1 July 2014. Time to death owing to any cause was calculated as the time from inclusion until death or last follow-up before 1 July 2014. For the 1016 patients included in these analyses, there were 122 breast cancer events, out of which 76 were distant metastases. A total of 97 patients had died during follow-up, and 39 of these had no reported breast cancer event.

To evaluate if preoperative smoking status was representative, changes in smoking status during the first postoperative year were assessed using the reported smoking status at the preoperative visit and follow-up visits after 3–6 months and 1 year. Missing data were handled according to the last observation carried forward method. In the case of missing data, the reported smoking status

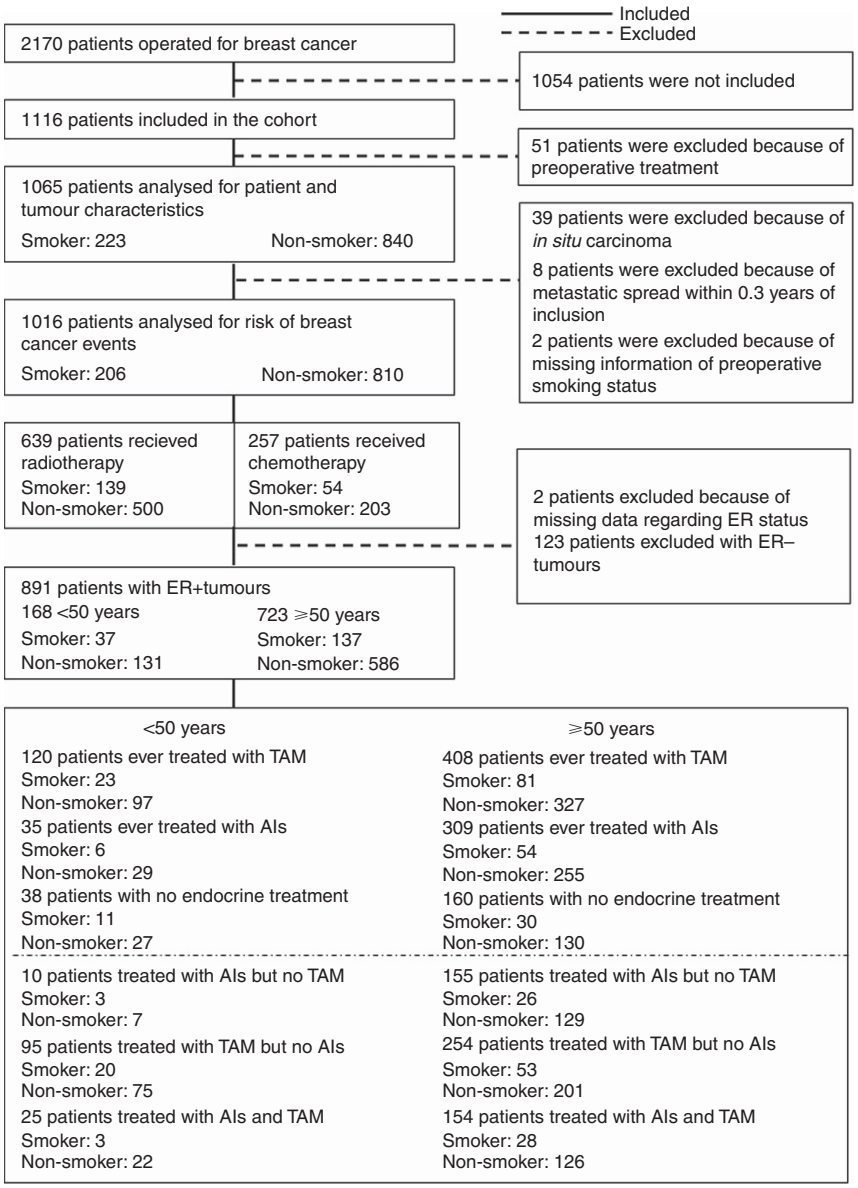

Figure 1. Flowchart of patients in different analyses in relation to their preoperative smoking status. AIs = aromatase inhibitors; ER = oestrogen receptor; TAM = tamoxifen.

from the previous visit was carried forward if the total follow-up time was longer than 0.5 years for the visit after 3–6 months ($n = 61$) and 1.0 year for the visit after 1 year ($n = 34$). Therefore, if patients had died from non-breast cancer-related causes before 0.5 years postoperatively ($n = 1$) or 1 year postoperatively ($n = 1$), they were not included in the analyses of these visits. Also, patients who were alive and event-free but had a follow-up shorter than 0.5 years or 1 year because they had not been to the visit at 3–6 months ($n = 12$) or 1 year postoperatively ($n = 27$) were not included in the analysis of smoking status at the respective visit. If a breast cancer event occurred before a visit, no last observation carried forward or data collected at this visit were included in the analysis for the visit 3–6 months postoperatively ($n = 2$) and for the visit 1 year postoperatively ($n = 11$). One patient had an event on the day of her visit. The data from this visit was included.

To calculate risk of breast cancer events, distant metastases, or death, Kaplan–Meier estimates were used. Cox's regressions were used to obtain adjusted hazard ratios (adjHRs) with 95% confidence intervals (CIs). Adjustments were made for invasive tumour size $\geqslant 21$ mm or muscle or skin involvement (yes/no), axillary lymph node involvement (yes/no), histological grade III (yes/no), positive ER status (yes/no), age (continuous), and body mass index (BMI) $\geqslant 25\,kg\,m^{-2}$ (yes/no). Further adjustments for treatment factors included ever treatment with radiation therapy (yes/no), chemotherapy (yes/no), TAM (yes/no), and AIs (yes/no).

The questionnaire included questions regarding menopausal status. Owing to risk of misclassification of menopausal status for hormonal therapy users and patients with previous gynaecological surgeries, age $\geqslant 50$ years was used as a proxy variable for postmenopausal status.

A P-value < 0.05 was considered statistically significant, and all P-values were two-sided. Nominal P-values are presented without adjustment for multiple testing.

RESULTS

Patient and tumour characteristics. Out of the 1065 patients included in the study, 223 (21%) reported to be smokers at the time

of the preoperative visit (Table 1). The smokers were in general younger, had a lower body weight, BMI, and preoperative total breast volume. Moreover, the smokers had fewer children, were significantly younger at their first full-term pregnancy, and were more likely to have used oral contraceptives. Tumour characteristics were similar between smokers and non-smokers except for hormone receptor status. Smokers had more often hormone receptor-negative tumours compared with non-smokers (Table 2).

Reported smoking status over time. Risk of breast cancer events in relation to smoking status was analysed in the 1016 patients with invasive tumours and no distant metastases were detected on postoperative metastases screen within 0.3 years of surgery. Of these, 206 were considered smokers and 810 were not considered smokers at the time of the preoperative visit (Figure 1). Figure 2 shows how the smoking habits of these patients changed during the first postoperative year. Less than 1% of the 810 preoperative non-smokers reported smoking at either the 3–6 months or 1-year postoperative visit, whereas about 10% of the patients who smoked preoperatively reported not to smoke during the follow-up visits. Thus, the majority of the patients did not switch smoking status.

Smoking and the risk of breast cancer events and death in different treatment groups. Patients were followed for up to 11 years, and the median follow-up time was 5.1 years (interquartile range (IQR): 3.0–7.2) for the 855 patients who were still alive and at risk of breast cancer events. Overall, there was no significant association between smoking at the preoperative visit and risk of breast cancer events (log rank, $P=0.14$; adjHR: 1.45; 95% CI: 0.95–2.20) adjusted for patient and tumour characteristics (Figure 3A). In all patients, smoking was associated with a two-fold increased risk for death owing to any cause (log rank, $P=0.037$; adjHR: 2.03; 95% CI: 1.29–3.21). No association was observed between smoking and risk of breast cancer events among the 257 chemotherapy-treated patients (log rank, $P=0.69$) (Figure 3B). Among the 639 radiotherapy-treated patients, there was a tendency towards an increased risk of a breast cancer event among smokers (log rank, $P=0.08$; adjHR: 1.71; 95% CI: 1.02–2.88) (Figure 3C).

Survival analysis in relation to endocrine treatment was restricted to the 891 patients with ER+ tumours. Among the patients younger than 50 years ($n=168$), there was no significant association between smoking and prognosis neither among the 120 patients who had ever received TAM nor among the 35 patients who had ever received AIs (all log rank Ps $\geqslant 0.21$).

For the 309 AI-treated patients $\geqslant 50$ years, smoking was significantly associated with an increased risk of breast cancer events (log rank, $P=0.005$; adjHR: 2.97; 95% CI: 1.44–6.13) (Figure 4A), distant metastasis (log rank, $P=0.002$; adjHR: 4.19; 95% CI: 1.81–9.72) (Figure 4B), and death (log rank, $P=0.003$; adjHR: 3.52; 95% CI: 1.59–7.81) (Figure 4C). The absolute risk for breast cancer events was 17.5/1000 person-years among non-smokers and 48.2/1000 person-years for smokers. For the 408 TAM-treated patients $\geqslant 50$ years, smoking was not significantly associated with risk for breast cancer events (log rank, $P=0.39$) (Figure 4D). Among TAM-treated patients never treated with AIs, there was no association between preoperative smoking status and risk for breast cancer events (log rank, $P=0.51$).

Further adjustments for other types of treatment modalities than the one selected did not materially change the result, except for the AI-treated patients where the adjHRs increased after further adjustments for TAM, chemotherapy, and radiotherapy.

As smoking appeared to have the strongest association in the AI-treated patients, stratification according to AI treatment was performed for the patients treated with radiotherapy, where a weak association between smoking and breast cancer events was found. Here, there was a four-fold risk for events in the 233 radiotherapy-treated patients who had received AIs (log rank, $P<0.001$; adjHR: 4.13; 95% CI: 1.66–10.26). No association between smoking and events was observed in the 406 radiotherapy-treated patients who had not received AIs (log rank, $P=0.94$).

Table 1. Patient characteristics in relation to smoking status at the preoperative visit

	All		Smoker at the preoperative visit[a]		
	Median (IQR) or %		Median (IQR) or %		
	$n=1065$	Missing	Yes $n=223$ (21.0%)	No $n=840$ (79.0%)	*P*-value
Age at diagnosis (years)	61.3 (52.3–68.1)	0	59.0 (51.3–65.4)	61.9 (52.7–69.0)	**<0.001**
Year of birth	1946 (1940–1955)	0	1948 (1943–1956)	1945 (1940–1954)	**0.001**
Weight (kg)	69.0 (62.0–78.0)	27	66.6 (60.0–76.0)	70.0 (62.0–78.5)	**<0.001**
Height (m)	1.65 (1.62–1.70)	27	1.65 (1.62–1.69)	1.65 (1.62–1.70)	0.76
BMI (kg m^{-2})	25.1 (22.5–28.3)	29	24.4 (21.7–27.2)	25.2 (22.7–28.7)	**<0.001**
Waist-to-hip ratio	0.86 (0.81–0.90)	39	0.87 (0.82–0.90)	0.85 (0.81–0.90)	0.09
Total breast volume (ml)[b]	1000 (650–1500)	167	800 (600–1300)	1000 (700–1600)	**<0.001**
Age at menarche (years)	13 (12–14)	6	13 (12–14)	13 (12–14)	0.55
Parous	87.9%	1	85.7%	88.6%	0.24
Parity	2 (1–3)	1	2 (1–2)	2 (1–3)	0.06
Age at first full-term pregnancy (years)[c]	25 (22–28)	136	23 (20–26)	25 (22–28)	**<0.001**
Alcohol abstainer	10.5%	7	7.2%	11.4%	0.07
Ever treated for menopausal symptoms	44.4%	3	39.0%	46.0%	0.06
Ever use of oral contraceptives	70.7%	1	78.0%	68.8%	**0.007**

Abbreviations: BMI = body mass index; IQR = interquartile range.
[a]Smoking status missing for two patients.
[b]In patients without previous breast surgery.
[c]In parous patients. The bold numbers indicate statistical significance.

Table 2. Tumor characteristics in relation to smoking status at the preoperative visit

	All	Smoker at the preoperative visit[a]		
	Number and %	Number and %		
	n = 1065	Yes *n* = 223 (21%)	No *n* = 840 (79%)	*P*-value
Invasive tumour size				$P_{trend} = 0.19$
In situ	39 (3.7%)	13 (5.8%)	26 (3.1%)	
1–20 mm	740 (69.5%)	155 (69.5%)	584 (69.5%)	
21–50 mm	269 (25.3%)	51 (22.9%)	217 (25.8%)	
51 or larger	15 (1.4%)	4 (1.8%)	11 (1.3%)	
Muscle or skin involvement	2 (0.2%)	0 (0.0%)	2 (0.2%)	
⩾21 or muscle or skin involvement	286 (26.9%)	55 (24.7%)	230 (27.4%)	$P = 0.42$
Missing	0	0	0	
No. of involved axillary lymph nodes				$P_{trend} = 0.55$
0	665 (62.6%)	146 (65.5%)	519 (61.9%)	
1–3	307 (28.9%)	57 (25.6%)	249 (29.7%)	
4+	91 (8.6%)	20 (9.0%)	70 (8.4%)	
Axillary node involvement (yes)	398 (37.4%)	77 (34.5%)	319 (38.1%)	$P = 0.33$
Missing	2	0	2	
Histological grade				$P_{trend} = 0.45$
I	252 (23.8%)	66 (29.9%)	186 (22.2%)	
II	519 (49.0%)	89 (40.3%)	430 (51.4%)	
III	288 (27.2%)	66 (29.9%)	220 (26.3%)	
Histologic grade III (Yes)	288 (27.2%)	66 (29.9%)	220 (26.3%)	$P = 0.29$
Missing	6	2	4	
Hormone receptor status				
ER+	899 (87.1%)	176 (82.2%)	722 (88.5%)	**$P = 0.02$**
PgR+	728 (70.7%)	135(63.4%)	592 (72.7%)	**$P = 0.008$**
ER+ PgR+	722 (70.2%)	132 (62.0%)	589 (72.4%)	**$P = 0.003$**
ER+ PgR−	176 (17.1%)	43 (20.2%)	133 (16.3%)	$P = 0.18$
ER− PgR−	125 (12.1%)	35 (16.4%)	89 (10.9%)	**$P = 0.03$**
ER− PgR+	6 (0.6%)	3 (1.4%)	3 (0.4%)	$P = 0.11$[b]
Missing	36	10	26	
HER2 gene amplification[c]	86 (12.5%)	13 (9.6%)	72 (13.1%)	$P = 0.26$
Missing	377	87	290	

Abbreviations: ER = oestrogen receptor; HER2 = human epidermal growth factor 2; PgR = progesterone receptor tumours.
[a]Smoking status missing for two patients.
[b]Fisher's exact test.
[c]HER2 was routinely analysed first as of November 2005, and in patients younger than 70 years of age with invasive tumours. The bold numbers indicate statistical significance.

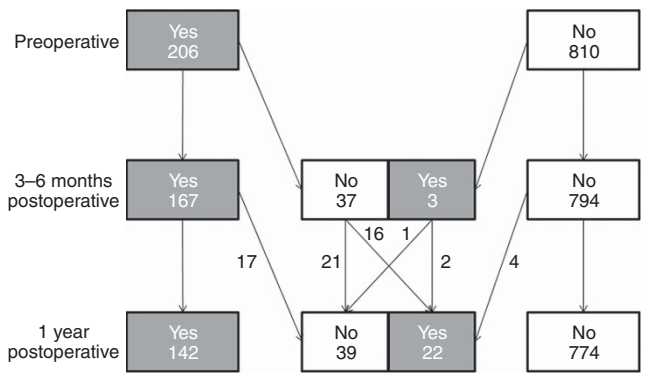

Figure 2. Flowchart of smoking status among alive and event-free patients using 'last observation carried forward'. Out of the 206 preoperative smokers, 21 patients (10.2%) reported no further smoking during the first postoperative year. Out of the 810 preoperative non-smokers, seven patients (<1%) reported smoking during the first postoperative year.

Similarly, after exclusion of AI-treated patients, there was no association between smoking and breast cancer events (log rank, $P = 0.98$) or distant metastasis (log rank, $P = 0.51$) in the remaining patients irrespective of age and ER status. However, there was a borderline significant increased risk for death due to any cause among patients who had not received AI treatment, but this was only found in the multivariable model (log rank, $P = 0.43$; adjHR: 1.82; 95% CI: 1.01–3.26).

DISCUSSION

The main finding of the present study was the increased risk of breast cancer events, distant metastasis, and death among AI-treated patients ⩾50 years who smoked at the preoperative visit compared with non-smokers. To our knowledge, this association has not been previously reported. Smoking was not associated with breast cancer events or distant metastases in other treatment groups.

In line with several other large studies including between 792 and 20 691 patients, current smoking was associated with a two-fold increased risk for death due to any cause (Manjer *et al*, 2000; Holmes *et al*, 2007; Braithwaite *et al*, 2012; Berube *et al*, 2014; Pierce *et al*, 2014; Seibold *et al*, 2014; Nechuta *et al*, 2016; Passarelli *et al*, 2016), with effect sizes ranging from 1.34 to 2.63. These studies also had access to data on former smoking history and five of them showed an increased risk for death also with former

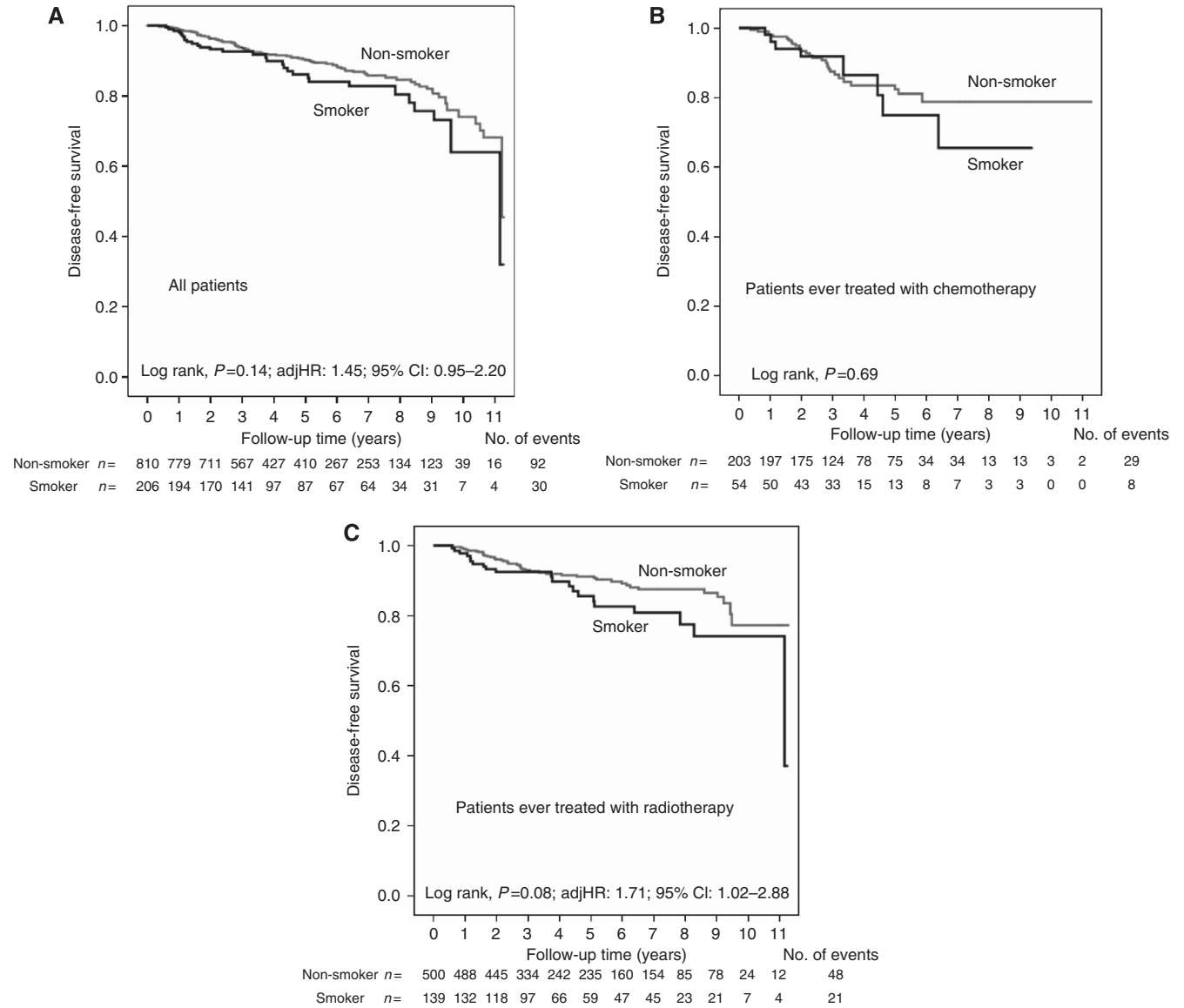

Figure 3. Kaplan–Meier estimates showing the association between preoperative smoking status and risk for breast cancer events. As this is an ongoing cohort, there are fewer patients with longer follow-up times. (**A**) There was no association among all patients. (**B**) There was no association among patients ever treated with chemotherapy. (**C**) Smoking was associated with a tendency towards an increased risk for breast cancer events among patients ever treated with radiotherapy. adjHR = adjusted hazard ratios; CI = confidence interval.

smoking (Braithwaite *et al*, 2012; Berube *et al*, 2014; Pierce *et al*, 2014; Nechuta *et al*, 2016; Passarelli *et al*, 2016). However, in two of these studies, this association was only found in former smokers with 20+ pack-years (Pierce *et al*, 2014; Nechuta *et al*, 2016). These two latter studies were partly based on the same study population and smoking was assessed on average 2 years after diagnosis, thus excluding early events. Only one other study examined current smoking in relation to all-cause mortality in different treatment groups. This study stratified according to TAM treatment, radiotherapy, and chemotherapy and reported no increased risk, but showed no data on AI treatment (Holmes *et al*, 2007). Their finding of no increased risk in patients treated with TAM, radiotherapy, or chemotherapy is in line with the results of the present study. Eight of these studies investigated breast cancer-specific survival (Manjer *et al*, 2000; Holmes *et al*, 2007; Braithwaite *et al*, 2012; Berube *et al*, 2014; Pierce *et al*, 2014; Seibold *et al*, 2014; Nechuta *et al*, 2016; Passarelli *et al*, 2016), of which five reported a statistically significant increased risk for current smokers ranging between 1.25 and 2.14 (Manjer *et al*, 2000; Braithwaite *et al*, 2012; Pierce *et al*, 2014; Nechuta *et al*, 2016; Passarelli *et al*, 2016). Risk for recurrence with current smoking ranged from 1.05 to 1.41 in four studies, but was significant only in the one study with the highest estimate (Pierce *et al*, 2014) and not in the three other studies (Holmes *et al*, 2007; Seibold *et al*, 2014; Nechuta *et al*, 2016). In the study by Nechuta *et al* (2016), former smokers with 20+ pack-years had a statistically increased risk for recurrence. Their study examined late recurrences 5+ years postdiagnosis and only included patients with ER+ tumours (Nechuta *et al*, 2016). In the present study, former smokers were grouped with never smokers and this may have attenuated the results. None of the referenced eight former studies took AI treatment into account and the vast majority of patients were included before routinely available AI treatment.

There could be several mechanisms behind the results in the present study of a worse short-term prognosis in AI-treated patients. In line with other studies (Albanes *et al*, 1987; Barrett-Connor and Khaw, 1989; Molarius *et al*, 1997; Holmes *et al*, 2007; Abramowitz *et al*, 2010; Braithwaite *et al*, 2012; Kwok *et al*, 2012;

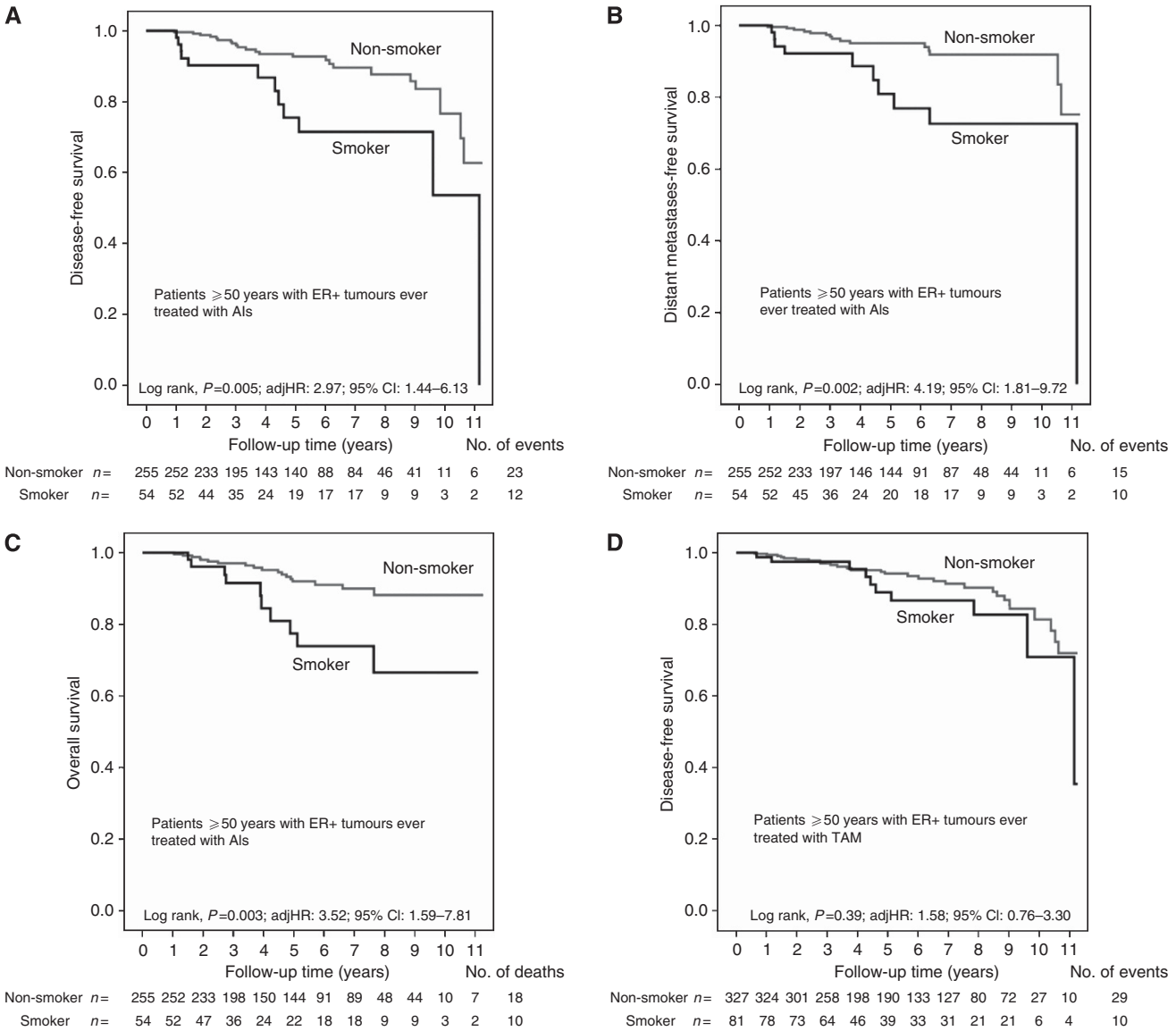

Figure 4. Kaplan–Meier estimates showing the association between preoperative smoking status and risk of breast cancer events, distant metastases, and death due to any cause among patients ⩾50 years with ER+ tumors. As this is an ongoing cohort, there are fewer patients with longer follow-up times. (**A**) Smoking was associated with a three-fold increased risk for breast cancer events among AI-treated patients. (**B**) Smoking was associated with a four-fold increased risk for distant metastases among AI-treated patients. (**C**) Smoking was associated with a three-fold increased risk of death due to any cause among AI-treated patients. (**D**) There was no association between smoking and risk for breast cancer events among TAM-treated patients. AdjHR = adjusted hazard ratios; AIs = aromatase inhibitors; CI = confidence interval; ER = oestrogen receptor; TAM = tamoxifen.

Berube *et al*, 2014; Huzell *et al*, 2015), several patient characteristics that may influence prognosis differed between smokers and non-smokers in the present study. We have previously reported higher frequency of smoking with increasing pre- and post-operative alcohol intake in the present cohort (Simonsson *et al*, 2014). However, alcohol intake was not associated with increased risk for events in any treatment group in this cohort and cannot explain the association between smoking and risk for events in AI-treated patients. Smokers had lower BMIs but a tendency towards larger waist-to-hip ratios and smaller breast volumes than non-smokers. These anthropometric factors have been associated with a more androgenic profile (Björntorp, 1997; Baglietto *et al*, 2009) that may influence AI response (Morris *et al*, 2001). Smoking may also be associated with other patient characteristics that were not assessed in this study such as patterns of physical activity that may influence prognosis (Nechuta *et al*, 2016).

Smoking was also associated with hormone receptor-negative tumours in the present cohort, whereas results from other studies are conflicting. A cohort of over 2000 breast cancer cases found no association between smoking and hormone receptor status (Braithwaite *et al*, 2012). A large cohort of 148 000 women reported an increased risk for ER+ cancer but no association with incident triple-negative cancer with over 40 pack-years of smoking (Kabat *et al*, 2011), which is in line with another large cohort of 117 000 women who reported smoking to be weakly associated with development of ER+ tumours (London *et al*, 1989). Conversely, smoking was significantly associated with development of hormone receptor-negative breast cancer in a South Swedish cohort of 10 000 women (Manjer *et al*, 2001). In Sweden, tumours are considered ER+ when >10% of the nuclei are stained, whereas other countries have a cutoff of >1%. Exact ER levels were unavailable in the present study, but according to a review, it

remains unclear to what extent hormone receptor levels impact on treatment response (Rastelli and Crispino, 2008).

Nicotine and tobacco alkaloids have been shown to inhibit oestrogen synthesis via the aromatase enzyme *in vitro* (Barbieri *et al*, 1986; Kadohama *et al*, 1993). Tumours that develop in smokers may already be resistant to AIs. In the present study, there were no data regarding smoking history. Former smokers were analysed as non-smokers. If smoking renders the tumour AI-resistant, this would have led to a bias towards the null. Data on former smoking would have enabled analyses of whether the tumours were already resistant to AIs irrespective of smoking status during AI treatment. If the tumour were AI-resistant, smokers could be offered TAM, as smoking was not associated with prognosis in TAM-treated patients. Cigarette smoke may also interact with therapy through upregulation of cytochrome *P*450 enzymes such as CYP1A2 that is involved in both metabolism of oestrogens and AIs (Grimm and Dyroff, 1997; Schrenk *et al*, 1998; Tsuchiya *et al*, 2005; Kamdem *et al*, 2011). Moreover, *CYP1A2* genotypes predicted short-term prognosis in AI-treated patients from a subset of this cohort (Simonsson *et al*, 2016). If cigarette smoke interacts with AIs, smokers assigned to AIs should be encouraged to quit. As only 10% of the preoperative smokers in the present study quit during the first year of follow-up, evaluation of smoking cessation was not possible.

Smokers tended to have a somewhat shorter duration of endocrine treatment (data not shown), and this may in part explain the increased risk of events among AI-treated smokers. Previous work from the same cohort reported that preoperative smokers are more likely to be non-adherent to endocrine therapy (Markkula *et al*, 2012b). However, this does not explain why there was no association between smoking and risk for events in TAM-treated patients.

This study has some limitations. No data on former smoking habits, socioeconomic status, or exact ER levels were collected. Also, the mechanisms behind the association between smoking and worse prognosis in AI-treated patients remain to be elucidated. A strength of the present study was that it is population-based, as patients were not referred to other hospitals for surgery. The majority of the female patients with primary breast cancer that fit the inclusion criteria participated in the study, and the main reason for non-participation was lack of available research nurses, where non-inclusion was unrelated to characteristics of the patients or their type of tumours. Approximately 5% of patients had an unclear diagnosis at the time of surgery and were therefore not included (Lundin *et al*, 2011). The included patients were comparable to all operated female patients with respect to age but had slightly higher frequency of ER+ and PgR+ tumours. No data were available on socioeconomic status or other tumour characteristics.

Another strength was that information on smoking was collected from questionnaires both pre- and postoperatively and not from patients' charts. As it was a prospective study, the risk for bias in the smoking variable due to survival or recall bias was minimised.

In conclusion, preoperative smoking was only associated with an increased risk for breast cancer events and distant metastasis among AI-treated patients. If confirmed, smoking status should be taken into consideration when selecting endocrine therapy.

ACKNOWLEDGEMENTS

We thank research nurses Anette Ahlin Gullers, Anita Schmidt Casslén Monika Meszaros, Maj-Britt Hedenblad, Karin Henriksson, Anette Möller, Helén Thell, Jessica Åkesson, and Linda Ågren. We also thank Erika Bågeman, Maria Henningson, and Maria Hjertberg for data entry. We acknowledge Klaus Bjerregaard and Ann-Sofi Hörstedt for providing statistics on breast cancer patients operated in the Skåne University Hospital in Lund. This work was supported by grants from The Swedish Cancer Society (CAN2014/465); the Swedish Research Council (K2012-54X-22027-01-3); the Medical Faculty at Lund University; the Mrs Berta Kamprad Foundation (BKS19/2014, BKS27/2015); the Gunnar Nilsson Foundation; the Swedish Breast Cancer Group (BRO); the South Swedish Health Care Region (Region Skåne ALF 10622); Konung Gustaf V:s Jubileumsfond; and the Lund Hospital Fund. The funding agencies had no role in design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; nor the decision to submit the manuscript for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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