



Miscellaneous

# Negative controls to detect uncontrolled confounding in observational studies of mammographic screening comparing participants and non-participants

Mette Lise Lousdal <sup>1</sup>\* Timothy L Lash,<sup>2,3</sup> W Dana Flanders,<sup>2,3</sup> M Alan Brookhart,<sup>4</sup> Ivar Sønbo Kristiansen,<sup>5</sup> Mette Kalager<sup>5,6</sup> and Henrik Støvring<sup>1</sup>

<sup>1</sup>Department of Public Health, Aarhus University, Aarhus, Denmark, <sup>2</sup>Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA, <sup>3</sup>Winship Cancer Institute, Emory University, Atlanta, GA, USA, <sup>4</sup>Department of Population Health Sciences, Duke University, Durham, NC, USA, <sup>5</sup>Department of Health Management and Health Economics, Oslo University, Oslo, Norway and <sup>6</sup>Clinical effectiveness research group, Oslo University Hospital, Oslo, Norway

\*Corresponding author. Department of Public Health, Aarhus University, Bartholins Allé 2, DK-8000 Aarhus C, Denmark. E-mail: lousdal@ph.au.dk

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## Abstract

**Background:** When comparing mammography-screening participants and non-participants, estimates of reduction in breast-cancer mortality may be biased by poor baseline comparability. We used negative controls to detect uncontrolled confounding.

**Methods:** We designed a closed cohort of Danish women invited to a mammography-screening programme at age 50–52 years in Copenhagen or Funen from 1991 through 2001. We included women with a normal screening result in their first-invitation round. Based on their second-invitation round, women were divided into participants and non-participants and followed until death, emigration or 31 December 2014, whichever came first. We estimated hazard ratios (HRs) of death from breast cancer, causes other than breast cancer and external causes. We added dental-care participation as an exposure to test for an independent association with breast-cancer mortality. We adjusted for civil status, parity, age at first birth, educational attainment, income and hormone use.

**Results:** Screening participants had a lower hazard of breast-cancer death [HR 0.47, 95% confidence interval (CI) 0.32, 0.69] compared with non-participants. Participants also had a lower hazard of death from other causes (HR 0.43, 95% CI 0.39, 0.46) and external causes (HR 0.35, 95% CI 0.23, 0.54). Reductions persisted after covariate adjustment. Dental-care participants had a lower hazard of breast-cancer death (HR 0.75, 95% CI 0.56, 1.01), irrespective of screening participation.

**Conclusions:** Negative-control associations indicated residual uncontrolled confounding

when comparing breast-cancer mortality among screening participants and non-participants.

**Key words:** Breast cancer, mammography screening, uncontrolled confounding, negative controls

### Key Messages

- Comparisons of screening participants and non-participants may suffer from poor baseline comparability, resulting in uncontrolled confounding.
- Negative-control exposures and outcomes may detect residual confounding by exploiting similarity of confounding structures.
- We found that mammography-screening participants were less likely than non-participants to die from causes other than breast cancer and from external causes.
- Dental-care participants were less likely than dental non-participants to die from breast cancer, irrespective of screening participation.
- Negative-control associations suggest uncontrolled confounding, even after adjustment for several well-known confounders.

## Introduction

In observational studies of mammography screening, the reduction in breast-cancer mortality can be estimated from intent-to-screen or actual screening-participation comparisons. Results from the first studies will be diluted due to non-compliance, but likely unbiased because of the random assignment of exposure. The latter studies<sup>1–3</sup> estimate efficacy, but are challenged by the non-random assignment of exposure: participants and non-participants may have different baseline risks of breast-cancer death.<sup>4,5</sup> Therefore, they rely on statistical-confounding adjustment to render the baseline risk comparable between participants and non-participants. Negative controls are an epidemiologic tool to detect potential residual confounding after adjustment for measured confounders.<sup>6</sup> Negative controls are causally unrelated factors, which have similar bias structures as the main association of interest. A negative-control analysis is expected to produce a result of no association. When it does not, the main association may be biased by the same structures that caused the negative-control experiment to fail. An ideal negative-control *outcome* and the exposure of interest share the same set of common causes as the exposure and outcome of interest.<sup>6</sup> Likewise, an ideal negative-control *exposure* and the outcome of interest share the same set of common causes as the exposure and outcome of interest.<sup>6</sup> We hypothesized that screening participants are healthier than non-participants and, therefore, already at baseline have a lower risk of breast-cancer death. We therefore used proxies for better health as negative-control outcomes and

proxies for healthier behaviour as negative-control exposures.

We aimed to evaluate uncontrolled confounding when estimating the breast-cancer-mortality reduction of mammography-screening participants compared with non-participants. As negative-control *outcomes*, we used death from causes other than breast cancer and from external causes such as accidents, intentional self-harm and assaults. As a negative-control *exposure*, we used dental-care participation.

## Methods

### Study design and setting

We constructed a closed cohort of Danish women invited to participate in a mammography-screening programme every other year in Copenhagen or Funen.<sup>7</sup> Healthcare in Denmark is generally tax-funded and free of charge, whereas dental care has approximately 80% patient co-payments.<sup>8</sup> Dentists invite their patients for regular examinations every 6–12 months.<sup>9</sup> Registration as a patient at a dental clinic is an individual responsibility. Approximately 65% of individuals aged 50–59 years attended dental care in 1990.<sup>8</sup>

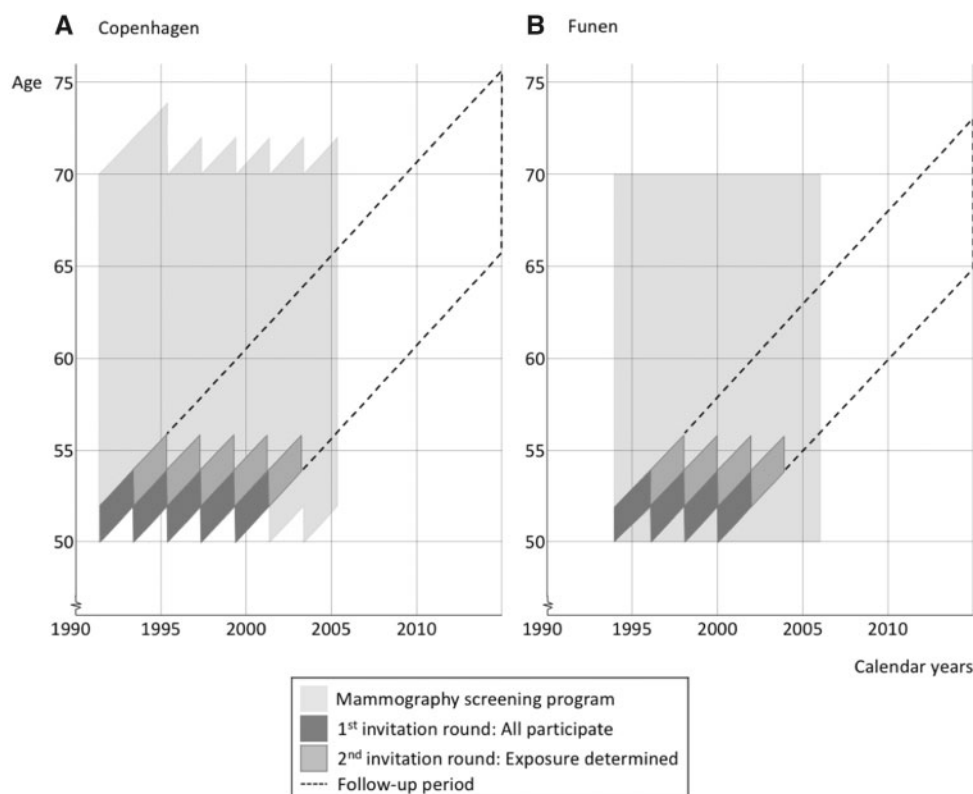
### Study population and data sources

We included women invited at age 50–52 years at the start of a 2-year mammography-screening round in Copenhagen

(April 1991 to March 2001) or Funen (November 1993 to December 2001). We only included women who had been living in Denmark since the age of 30 years without breast cancer (ICD10-code: D05 or C50) before enrolment in the screening programme. Further, only women who had participated in their first-personal-invitation round with a normal screening result were included. If the first or second screening exam was delayed >1 year after the screening round officially ended, women were not included ( $n = 15$ ). Follow-up started on the date of the second screening exam for participants. For non-participants in Copenhagen, follow-up started on the administratively scheduled date of the missed second exam. When the administratively scheduled date was missing, we mimicked the way it was assigned in Copenhagen, i.e. we set follow-up to start on the date of their first screening exam adding 2 years for non-participants in Funen and a few non-participants in Copenhagen ( $n < 5$ ). We followed women until death, emigration or end of follow-up (31 December 2014), whichever came first (Figure 1).

We linked data from publicly available registries using the unique personal-identification number assigned to all individuals living in Denmark.<sup>10</sup> Data on participation in mammography screening, screen-detected and interval cancers were retrieved from mammography-screening registers

in Copenhagen and Funen from the Danish Data Archive.<sup>11–13</sup> Information on breast cancer was obtained from the Danish Cancer Registry<sup>14</sup>; civil status and dates of emigration, immigration and death from the Danish Civil Registration System<sup>10</sup>; and cause of death from the Danish Register of Causes of Death.<sup>15</sup> Data on parity and age at first birth were obtained from the Fertility Database<sup>16</sup>; years of education and annual income from Statistics Denmark; and prescriptions of oestrogen and progesterone–oestrogen combination drugs (Anatomical therapeutic chemical groups: G03C and G03F) from the Danish National Prescription Registry.<sup>17</sup> Indication for prescriptions was unknown, but all women were aged >45 years at time of redemption and therefore these drugs were most likely for menopausal symptoms. Data on dental care were obtained from the Danish National Health Service Register, which contains information on services for public-sector reimbursements of dentists.<sup>18</sup> Due to Statistic Denmark's confidentiality regulations, in cases in which a specific risk factor was missing for fewer than five individuals, we could not report the actual number. These individuals were excluded from the study ( $n = 8$ ). Remaining individuals with missing information on educational attainment ( $n = 401$ ) were included in the unadjusted analyses, but excluded in the adjusted analyses.



**Figure 1.** Study design outlining first and second personal-invitation rounds and follow-up periods for women invited for mammography screening in Copenhagen (A) and Funen (B), 1991–2014.

The Danish Data Protection Agency approved the study (Journal number 2014–41–2871).

### Study variables

All women had to participate in their first-invitation round with a normal screening result to ensure that women were free from breast cancer at baseline with no dormant cancers. Therefore, exposure was determined based on their second-invitation round, where we categorized women into participants and non-participants of mammography screening. All risk factors were assessed during the last year of their first-invitation round, except income, which was calculated as the average across the first and last years of the first round. Civil status was categorized as married, divorced/widowed or unmarried. We created an indicator variable for nulliparity. Parity was categorized into 1, 2 or >2 children and age at first birth into <20, 20–24, 25–29 or ≥30 years. Years of education were categorized into ≤9, 10–14 or ≥15 years corresponding to elementary, medium long or higher education. Average annual income was categorized into <\$20 000, \$20 000–\$40 000 or >\$40 000 (exchange rate: 6.50 DKK per US dollar). We defined use of hormone drugs as at least two prescriptions of the same drug from 1995 onwards to identify persistent users and categorized women into ever vs never-users.

The primary outcome was death due to breast cancer registered as the underlying cause (ICD10-code: C50). The two negative-control *outcomes* were defined as death from causes other than breast cancer or from external causes such as accidents, intentional self-harm and assaults (ICD10-codes: V01–Y98). The negative-control *exposure* was defined as participation in dental care during the first personal-screening-invitation round. We counted the number of dental visits during the 2-year time slot and categorized dental-care exposure into participation (at least one visit) or non-participation. We included visits involving tooth scaling (payment codes = 1120, 1130, 1301, 1302) assuming that scaling is a proxy for healthier behaviour.

### Statistical analysis

We tabulated the number of deaths, person-time and risk factors across exposure categories and assessed numbers with missing information. We employed Cox regression with age as the underlying time scale to estimate hazard ratios (HRs) of breast-cancer mortality comparing mammography-screening participants with non-participants. We used two adjusted models: (i) a general model controlling for civil status, educational attainment and income and (ii) a breast-cancer-specific model controlling for parity, age at first birth, educational attainment, income and use of

hormone drugs. We assumed that most of the effect from civil status was mediated through reproductive history and therefore omitted civil status from the second model. To check the impact of choice of categorizations, we repeated the analyses using restricted cubic splines to flexibly adjust for education and income.

In the negative-control-*outcome* analysis, we replaced the outcome of breast-cancer death first with death from causes other than breast cancer and second with death from external causes. We repeated the unadjusted and the two adjusted Cox-regression models. Using the Fine–Gray hazard model<sup>19</sup> to take account of competing events did not notably change the results.

In the negative-control-*exposure* analysis, we maintained the comparison of breast-cancer mortality between mammography-screening participants and non-participants, but added dental-care participation as our main exposure. We repeated the unadjusted and the two adjusted Cox-regression models. To check for a dose–response relationship, we also employed the number of dental-care visits during the first-invitation round as a continuous variable. To check the robustness of results to coding-practice changes, we repeated the analyses changing the definition of dental care to either clinical examinations (payment codes = 1110, 1120, 1130, 1140) or tooth scaling and clinical examinations combined (payment codes = 1110, 1120, 1130, 1140, 1301, 1302).

To check the importance of setting the date for the missed second exam for non-participants in Funen, we performed two extreme scenario analyses changing the date to either the first day or the last day of the second-invitation round. Additionally, we repeated the analyses restricted to the Copenhagen cohort.

We implicitly assume that screening participation across a woman's lifetime can be determined based on behaviour during her second-invitation round. To check this assumption, we explored the relation between baseline participation and later participation. Later participation was operationalized as number of rounds with participation divided by the number of rounds with invitation, which varied due to age, diagnosis, death and other reasons. This resulted in a proportion of attended rounds per invited rounds. We compared this average proportion between screening participants and non-participants and between dental-care participants and non-participants. If baseline dental care shows an association with later participation in screening, dental care is not only a proxy for healthier behaviour, but also a proxy for later screening and may capture part of the true effect of mammography screening. This will to some extent invalidate the assumptions underlying the negative-control-exposure analysis, because the exposure of interest is not fully adjusted for

(Supplementary Figure 1, available as Supplementary data at IJE online).<sup>20</sup>

The proportional-hazards assumption was assessed using log-log plots. All statistical analyses were conducted in Stata, version 15 (StataCorp, College Station, TX, USA).

## Results

We included 36 608 women aged 50–52 years who had participated in their first-invitation round with a normal screening result. We excluded 401 women with missing information on education from the adjusted analyses. During follow-up, 179 and 1723 women were diagnosed with *in situ* or invasive breast cancer, respectively, and 219 women died of breast cancer. A total of 4099 women died of causes other than breast cancer, 152 of external causes, 19 of unknown causes and 265 emigrated. The median age at end of follow-up was 69 years (10<sup>th</sup>–90<sup>th</sup> percentile:

65–73 years). The median time between first and second screening exams was 2.0 years (10<sup>th</sup>–90<sup>th</sup> percentile: 1.8–2.1). Differences in risk factors between participants and non-participants are described in Table 1.

Participants had lower breast-cancer mortality [HR 0.47, 95% confidence interval (CI) 0.32, 0.69] compared with non-participants (Table 2). In the general model adjusted for civil status, education and income, participants still had lower breast-cancer mortality (HR 0.53; 95% CI 0.36, 0.79) compared with non-participants. In the breast-cancer-specific model adjusted for parity, age at first birth, education, income and hormone-drug use, participants also had lower breast-cancer mortality (HR 0.50; 95% CI 0.34, 0.74) compared with non-participants. Repeating the analyses using spline-based adjustment for education and income led to similar results.

In the negative-control-*outcome* analysis, screening participants had lower mortality from causes other than breast

**Table 1.** Deaths, person-time and risk factors across categories of mammography-screening participation among 36 608 Danish women participating in their first mammography-screening round at age 50–52 years in Copenhagen or Funen with a normal screening result, 1991–2001

	Mammography screening, second-invitation round			
	Participants ( <i>n</i> = 33 858)		Non-participants ( <i>n</i> = 2750)	
Breast-cancer deaths, <i>n</i>	188		31	
Person-time, years	500 689		38 431	
Risk factors				
Civil status				
Unmarried, <i>n</i> (%)	2541	(7)	333	(12)
Divorced/widowed, <i>n</i> (%)	7963	(24)	1073	(39)
Married, <i>n</i> (%)	23 354	(69)	1344	(49)
Children				
Nulliparous, <i>n</i> (%)	3352	(10)	400	(15)
Parous, <i>n</i> (%)	30 506	(90)	2350	(85)
Parity				
1 child, <i>n</i> (%)	5752	(19)	591	(25)
2 children, <i>n</i> (%)	16 579	(54)	1091	(46)
>2 children, <i>n</i> (%)	8175	(27)	668	(29)
Age at first birth, mean (sd)	23.4	(4.1)	23.4	(4.5)
Years of education, <sup>a</sup> mean (sd)	11.2	(3.2)	11.1	(3.4)
Average annual income in USD, mean (sd)	30 040	(16 682)	29 019	(19 681)
Hormone-drug use				
Ever, <i>n</i> (%)	9659	(29)	616	(22)
Never, <i>n</i> (%)	24 199	(71)	2134	(78)
Negative-control outcomes <sup>b</sup>				
Causes other than breast cancer, <i>n</i>	3472		627	
External causes, <i>n</i>	125		27	
Negative-control exposure: Dental care, first-invitation round				
Participants, <i>n</i> (%)	26 456	(78)	1723	(63)
Non-participants, <i>n</i> (%)	7402	(22)	1027	(37)

<sup>a</sup>401 with missing information.

<sup>b</sup>19 with missing cause of death.

**Table 2.** Relative hazards of breast-cancer mortality among 36 608 Danish women participating in their first mammography-screening round at age 50–52 years in Copenhagen or Funen with a normal screening result, 1991–2014

	Unadjusted model ( <i>n</i> = 36 608)		General model ( <i>n</i> = 36 207)		Breast-cancer-specific model ( <i>n</i> = 36 207)	
	HR	95% CI	HR	95% CI	HR	95% CI
Mammography screening, second-invitation round						
Participants	0.47	(0.32, 0.69)	0.53	(0.36, 0.79)	0.50	(0.34, 0.74)
Non-participants	1.00	Referent	1.00	Referent	1.00	Referent
Civil status						
Unmarried			1.08	(0.64, 1.83)		
Divorced/widowed			1.29	(0.95, 1.74)		
Married			1.00	Referent		
Children						
Nulliparous					1.46	(0.83, 2.57)
Parous					1.00	Referent
Parity						
1 child					1.00	Referent
2 children					1.04	(0.70, 1.55)
>2 children					1.08	(0.69, 1.70)
Age at first birth						
<20 years					1.37	(0.95, 1.97)
20–24 years					1.00	Referent
25–29 years					1.25	(0.86, 1.82)
≥30 years					1.12	(0.59, 2.13)
Years of education						
≤9 years			0.98	(0.73, 1.32)	0.96	(0.71, 1.31)
10–14 years			1.00	Referent	1.00	Referent
≥15 years			1.10	(0.70, 1.72)	1.07	(0.68, 1.68)
Average annual income in USD						
<20 000			1.56	(1.16, 2.10)	1.54	(1.14, 2.07)
20 000–40 000			1.00	Referent	1.00	Referent
>40 000			0.65	(0.41, 1.03)	0.65	(0.40, 1.03)
Hormone-drug use						
Ever					1.50	(1.12, 2.00)
Never					1.00	Referent

HR, hazard ratio; CI, confidence interval.

cancer (HR 0.43; 95% CI 0.39, 0.46) compared with non-participants (Table 3). In the adjusted models, the lower mortality persisted, but was attenuated. In addition, participants had lower mortality from external causes (HR 0.35; 95% CI 0.23, 0.54) compared with non-participants, which persisted, albeit attenuated, in the general model.

In the negative-control-exposure analysis, dental participants had lower breast-cancer mortality (HR 0.75, 95% CI 0.56, 1.01) compared with dental non-participants (Table 4). Screening participants also retained lower breast-cancer mortality (HR 0.49; 95% CI 0.33, 0.72) compared with screening non-participants. When adjusting for risk factors, the lower breast-cancer mortality among dental participants and among screening participants was attenuated. In the dose-response analysis, an extra dental

visit during the first-invitation round decreased the hazard of breast-cancer death irrespective of screening participation, both in the general model (HR 0.90, 95% CI 0.83, 0.97) and the breast-cancer-specific model (HR 0.89, 95% CI 0.82, 0.96). Changing the definition of included dental-care codes led to similar results (see Supplementary Table 1, available as Supplementary data at *IJE* online).

When setting the date of the missed second exam to the first day of the second-invitation round, HRs for screening participants were lower in the general (HR 0.48, 95% CI 0.33, 0.70) and breast-cancer-specific (HR 0.46, 95% CI 0.31, 0.67) models. When setting the date to the last day of the second-invitation round, HRs were similar to the main analysis in the general (HR 0.53, 95% CI 0.36, 0.79) and breast-cancer-specific (HR 0.50, 95% CI 0.34, 0.74)

**Table 3.** Relative hazards of mortality from negative-control outcomes among 36 608 Danish women participating in their first mammography-screening round at age 50–52 years in Copenhagen or Funen with a normal screening result, 1991–2014

	Death from causes other than breast cancer						Death from external causes (V01–Y98)					
	Unadjusted model ( <i>n</i> = 36 608)		General model ( <i>n</i> = 36 207)		Breast-cancer-specific model ( <i>n</i> = 36 207)		Unadjusted model ( <i>n</i> = 36 608)		General model ( <i>n</i> = 36 207)		Breast-cancer-specific model ( <i>n</i> = 36 207)	
	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Mammography screening, second-invitation round												
Participants	0.43	(0.39, 0.46)	0.49	(0.45, 0.53)	0.46	(0.42, 0.50)	0.35	(0.23, 0.54)	0.44	(0.29, 0.67)	0.37	(0.24, 0.57)
Non-participants	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent	1.00	Referent
Civil status												
Unmarried			1.89	(1.70, 2.09)					2.48	(1.44, 4.26)		
Divorced/widowed			1.66	(1.55, 1.78)					2.99	(2.11, 4.23)		
Married			1.00	Referent					1.00	Referent		
Children												
Nulliparous					1.37	(1.22, 1.54)					0.87	(0.48, 1.55)
Parous					1.00	Referent					1.00	Referent
Parity												
1 child					1.00	Referent					1.00	Referent
2 children					0.79	(0.72, 0.86)					0.43	(0.28, 0.66)
>2 children					0.74	(0.67, 0.82)					0.66	(0.41, 1.06)
Age at first birth												
<20 years					1.25	(1.15, 1.35)					0.92	(0.58, 1.47)
20–24 years					1.00	Referent					1.00	Referent
25–29 years					0.82	(0.75, 0.91)					0.89	(0.56, 1.43)
≥30 years					0.96	(0.83, 1.11)					1.05	(0.54, 2.04)
Years of education												
≤9 years			1.44	(1.34, 1.54)	1.40	(1.31, 1.50)			0.94	(0.66, 1.34)	1.00	(0.69, 1.44)
10–14 years			1.00	Referent	1.00	Referent			1.00	Referent	1.00	Referent
≥15 years			0.81	(0.72, 0.91)	0.84	(0.74, 0.95)			1.12	(0.66, 1.91)	1.09	(0.64, 1.87)
Average annual income (USD)												
<20 000			1.51	(1.41, 1.61)	1.44	(1.34, 1.54)			1.45	(1.00, 2.09)	1.30	(0.90, 1.87)
20 000–40 000			1.00	Referent	1.00	Referent			1.00	Referent	1.00	Referent
>40 000			0.75	(0.67, 0.84)	0.76	(0.69, 0.85)			0.50	(0.28, 0.89)	0.51	(0.29, 0.91)
Hormone-drug use												
Ever					1.01	(0.94, 1.08)					1.14	(0.79, 1.63)
Never					1.00	Referent					1.00	Referent

HR, hazard ratio; CI, confidence interval.

models. Changing the date had no effect on the negative-control associations. When restricted to the Copenhagen cohort, we found similar results, although the HRs for death from external causes were attenuated.

The average proportion of later-attended rounds per invited-screening rounds was 93% among screening participants vs 38% among screening non-participants and 91% among dental-care participants vs 82% among dental-care non-participants.

## Discussion

Mammography-screening participants had a lower hazard of death from causes other than breast cancer and from external causes than non-participants. In addition, dental-care participants had a lower hazard of breast-cancer death than dental non-participants, irrespective of screening participation. As these associations are causally implausible, this suggests that screening participants are healthier than non-participants already at baseline in ways that are

**Table 4.** Relative hazards of breast-cancer mortality associated with the negative-control exposure of dental-care participation, also adjusted for mammography participation as a potential mediator, among 36 608 Danish women participating in their first mammography-screening round at age 50–52 years in Copenhagen or Funen with a normal screening result, 1991–2014

	Unadjusted model ( <i>n</i> = 36 608)		General model ( <i>n</i> = 36 207)		Breast-cancer-specific model ( <i>n</i> = 36 207)	
	HR	95% CI	HR	95% CI	HR	95% CI
Dental care, first-invitation round						
Participants	0.75	(0.56, 1.01)	0.82	(0.60, 1.11)	0.80	(0.59, 1.08)
Non-participants	1.00	Referent	1.00	Referent	1.00	Referent
Mammography screening, second-invitation round						
Participants	0.49	(0.33, 0.72)	0.54	(0.37, 0.81)	0.52	(0.35, 0.77)
Non-participants	1.00	Referent	1.00	Referent	1.00	Referent
Civil status						
Unmarried			1.08	(0.64, 1.82)		
Divorced/widowed			1.26	(0.93, 1.71)		
Married			1.00	Referent		
Children						
Nulliparous					1.46	(0.83, 2.57)
Parous					1.00	Referent
Parity						
1 child					1.00	Referent
2 children					1.04	(0.70, 1.56)
>2 children					1.07	(0.68, 1.68)
Age at first birth						
<20 years					1.35	(0.94, 1.94)
20–24 years					1.00	Referent
25–29 years					1.26	(0.86, 1.82)
≥30 years					1.13	(0.59, 2.14)
Years of education						
≤9 years			0.95	(0.71, 1.28)	0.93	(0.69, 1.27)
10–14 years			1.00	Referent	1.00	Referent
≥15 years			1.10	(0.70, 1.73)	1.07	(0.68, 1.69)
Average annual income (USD)						
<20 000			1.54	(1.14, 2.08)	1.52	(1.13, 2.05)
20 000–40 000			1.00	Referent	1.00	Referent
>40 000			0.65	(0.41, 1.04)	0.65	(0.41, 1.04)
Hormone-drug use						
Ever					1.51	(1.13, 2.02)
Never					1.00	Referent

HR, hazard ratio; CI, confidence interval.

difficult or impossible to statistically control, leading to uncontrolled bias in observational associations.

### Strengths and limitations

The main strength of this study lies in the ability to adjust for several well-known risk factors due to the numerous and almost complete national registries. Although misclassification of cause of death occurs, we expect this to be unrelated to participation in mammography screening. Additional analyses showed that change of dental-care-coding

practices could not explain the observed association with breast-cancer death.

However, a study-design limitation arises because the administratively scheduled screening exam date is missing for non-participants in Funen. Extreme scenario analyses verified that this had little impact on the results. Also, due to the missing administratively scheduled exam date among non-participants, we could not identify the first-personal-invitation round for all women in Funen. For some women, the included invitation rounds corresponded to their second- and third-invitation rounds. We had to base



determination of screening participation on one round, but our analysis of later participation verified that the participation pattern is fairly stable across several rounds, which has also been found by others.<sup>21,22</sup> Preferably, we would have assessed screening participation dynamically throughout follow-up, but this was not possible with the current study design, since women become ineligible for a screening invitation after a diagnosis of breast cancer. We restricted the study population to women participating in their first-invitation round with a normal screening result to ensure that women were free from breast cancer at baseline with no dormant cancers. The exclusion of never-screened women limits the generalizability of our findings and at the same time strengthens our conclusions because all women participated in at least one round of screening. The baseline differences associated with never participating in screening may have been even stronger than the baseline differences identified among initial participants.

For the negative-control outcomes to be valid, mammography-screening participation should not directly cause differences in death from causes other than breast cancer or from external causes. Side effects from radiation and breast-cancer therapies may affect death from other causes, but this would increase and not reduce mortality among participants. For the negative-control exposure to be valid, dental-care participation should not directly affect breast-cancer mortality. Dental care is inconceivable to have any direct effect on breast-cancer mortality. However, according to the study design, later participation in mammography screening during follow-up is not assessed or adjusted. We found that dental-care participation during the first-personal-invitation round was associated with an increased likelihood of later mammography-screening participation. Therefore, part of the protective association between dental care and breast-cancer death may be mediated through later participation in mammography screening and represent a true benefit and not uncontrolled confounding. The relative hazards from screening participation remained almost unchanged in the negative-control-exposure analysis, which may result from the lack of adjustment for later mammography participation. The negative controls were pre-specified, although not pre-registered, based on subject-matter knowledge of the underlying causal and confounding structures. Initially, we also employed cervical-cancer-screening participation as a negative-control exposure, but the analyses showed severe effect modification, potentially arising from the small selected group of women who participated in cervical-cancer screening, but not in mammography screening.

Preferably, negative controls should share the same set of common causes as the exposure and outcome of interest. This assumption is more likely valid for general factors such as lifestyle, educational attainment, socio-economic status

and civil status than for breast-cancer-specific factors. We did not have information on family history of breast cancer or adherence to breast-cancer treatment. The negative controls might capture baseline differences in these unmeasured confounders if related to the underlying aspects of better health and healthier behaviour. Most likely, residual confounding remains in the negative-control analyses.

### Comparison with previous studies

The intractable bias, where participants already at baseline have a lower risk of the outcome than non-participants, has previously been termed ‘compliance bias’,<sup>23</sup> ‘prevention bias’,<sup>24</sup> ‘healthy adherer effect’<sup>25</sup> or ‘healthy user effect/bias’.<sup>26</sup> Negative-control-outcome analyses have shown that participants in mammography or cervical screening had lower mortality from cancers other than breast-cancer or all-cause deaths, respectively.<sup>27,28</sup> To our knowledge, negative-control exposures have not previously been employed, but studies have explored associations between different preventive measures. Both participation in mammography screening and dental care was associated with healthy behaviour such as non-smoking, exercise, seatbelt use, alcohol, diet and medical check-ups.<sup>29–32</sup>

Previous studies of mammography screening used purported correction factors to adjust for uncontrolled confounding. These factors compare the baseline risk of breast-cancer death of non-participants to participants and span from 0.64 to 1.36.<sup>4,5,33–37</sup> The diverging estimates question the generalizability across time and settings, but correction factors cannot be estimated once all women are invited for screening. Other studies employed quantitative-bias analysis,<sup>38,39</sup> but this approach cannot take account of interaction between multiple confounders. The negative controls cannot be used in a simple way to quantify the amount of residual confounding nor to estimate a correction factor.<sup>6</sup> Recent negative-control applications show potential to also partially correct for uncontrolled confounding.<sup>6,40–42</sup> However, we could not employ our negative-control exposure to conduct a partial correction, because dental care was associated with later participation in screening. This violates the underlying assumptions, because the exposure of interest is not fully adjusted for.

### Implications

In this closed cohort study of Danish women, we found that mammography-screening participants had about a 50% lower hazard of breast-cancer death than non-participants after statistical-confounding adjustment. However, negative-control associations revealed bias. The lower mortality observed in second-round participants could reflect a real benefit of

subsequent exams which they were more likely to attend, coupled with some benefit of attending the second exam, as well as uncontrolled confounding. The potential of negative controls to correct for uncontrolled confounding should be explored employing G-computation to solve the issue of time-varying screening exposure.<sup>43</sup> In the meantime, it is paramount that future mammography studies comparing participants and non-participants employ negative controls to check for uncontrolled confounding. Further, negative controls are relevant not only for studies of screening, but in general for observational studies based on electronic health records. Such studies typically have access to a variety of negative controls that should be employed to detect uncontrolled confounding.

## Supplementary Data

Supplementary data are available at *IJE* online.

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## Author Contributions

H.S. conceived the original study idea. M.L.L., H.S., T.L.L. and W.D.F. contributed to the study design. M.L.L. acquired the data, performed the data analyses and drafted the document. All authors contributed to the interpretation of the data, revised the manuscript critically for important intellectual content and gave final approval for the version to be published.

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