2016-11-29

Longitudinal fluctuation in mammographic percent density differentiates between interval and screen-detected breast cancer

Strand, Fredrik; Humphreys, Keith; Eriksson, Mikael; Li, Jingmei; Andersson, Therese M L; Törnberg, Sven; Azavedo, Edward; Shepherd, John; Hall, Per; Czene, Kamila

Int J Cancer. 2017 Jan 1;140(1):34-40.
http://doi.org/10.1002/ijc.30427
http://hdl.handle.net/10616/45408

Title: Longitudinal fluctuation in mammographic percent density differentiates between interval and screen-detected breast cancer

Authors and e-mail addresses: Fredrik Strand*[1,2](fredrik.strand@ki.se), Keith Humphreys[1,3] (keith.humphreys@ki.se), Mikael Eriksson[1] (mikael.eriksson@ki.se), Jingmei Li[1,4] (jingmei.li@ki.se), Therese ML Andersson[1] (therese.m-l.andersson@ki.se), Sven Törnberg[5] (sven.tornberg@sll.se), Edward Azavedo[6,7] (edward.azavedo@ki.se), John Shepherd[8] (john.shepherd@ucsf.edu), Per Hall[1] (per.hall@ki.se), Kamila Czene[1] (kamila.czene@ki.se)

Author affiliations (1-8):

1) Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
2) Department of Diagnostic Radiology, Karolinska University Hospital, Stockholm, Sweden
3) Swedish eScience Research Centre (SeRC), Karolinska Institutet, Stockholm, Sweden
4) Genome Institute of Singapore, Singapore, Singapore.
5) Department of Cancer Screening, Stockholm-Gotland Regional Cancer Centre, Stockholm, Sweden
6) Department of Diagnostic Radiology, Karolinska Institutet, Stockholm, Sweden
7) Department of Molecular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden
8) Department of Radiology and Biomedical Imaging, UCSF School of Medicine, University of California, San Francisco, California, United States

Corresponding author (*): Fredrik Strand

Address: Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, P.O. Box 281, SE-171 77, Stockholm, Sweden

E-mail: fredrik.strand@ki.se

Telephone: +46 8 52482363

Fax: +46 8314975

Novelty & Impact Statements

Interval breast cancers are more aggressive and are associated with a higher mortality than screen-detected cancers. Mammographic percent density is one of few known risk factors and can be automatically calculated based on screening mammograms. The authors of this study estimated long-term trends of mammographic density for each participating woman. They found that a measure of the fluctuation around the individual long-term trend was associated with an increased proportion of interval cancer. This is a novel and interesting finding that may enable us to better identify women at elevated risk - after validation in a screening population.

Abstract

Interval breast cancer (IC) has a more aggressive phenotype and higher mortality than screen-detected cancer (SDC). In this case-case study, we investigated whether the size of longitudinal fluctuations in mammographic percent density (PD fluctuation) was associated with the ratio of IC vs. SDC among screened women with breast cancer. The primary study population consisted of 1,414 postmenopausal breast cancer cases, and the validation population of 1,241 cases. We calculated PD fluctuation as the quadratic mean of deviations between actual PD and the long-term trend estimated by a mixed effects model. In a logistic regression model we examined the association between PD fluctuation and interval vs. screen-detected cancer including adjustments for PD at last screening, age at diagnosis, BMI and hormone replacement therapy. All statistical tests were two-sided. There were 385 IC and 1029 SDC in the primary study population, with PD fluctuations of 0.44 and 0.41 respectively (p=0.0309). After adjustments, PD fluctuation was associated with an increased ratio of IC vs. SDC, with an estimated per-standard deviation odds ratio of 1.17 (95% CI = 1.03 to 1.33), compared to 1.19 (95% CI = 1.04 to1.38) in the validation population. In screened women with breast cancer, high fluctuation in mammographic percent density was associated with an increased ratio of IC vs. SDC. Whether this is entirely related to a reduced mammographic detectability or to a biological phenotype promoting faster tumor growth remains to be elucidated.

INTRODUCTION

Interval breast cancer (IC) has a more aggressive phenotype and higher mortality than screen-detected cancer[1-6]. IC is defined as breast cancer that is detected after a negative screen but before the next regular visit or end of a normal screening interval - which in Sweden has a length of 18 to 24 months depending on age and county. In a review of 10 different studies, most of them Scandinavian, the proportion of ICs was 22 to 37 % in the regularly screened women[7].

IC has been shown to be associated with mammographic density [1, 8, 9], which is usually expressed as a percentage of the total breast area and called 'percent density' (PD). A higher PD means that there is more dense tissue in the breast that could potentially mask an incident tumor[10, 11]. The potential masking problem decreases as the woman ages due to a long-term trend of decreasing PD[12].

To minimize the risk that a subtle malignant change in the mammogram is missed it is good practice for radiologists to compare the current image with previous ones. We hypothesized that high fluctuation in density reflects large variations in mammographic appearance, which would increase the likelihood that a subtle malignant change passes unnoticed by the screening radiologist, i.e. that high fluctuation reduces mammographic detectability.

Our aim was to study a case-only cohort to examine whether a large fluctuation in PD between pre-diagnostic mammograms would be associated with an increased ratio of IC vs. SDC once a tumor has been initiated. PD fluctuation was calculated as a summary measure of the deviations between the actual PD measurements and the estimated individual long-term trend.

METHODS

Study Populations

We analyzed postmenopausal breast cancer cases in the Libro-1 cohort, which consists of women in the Stockholm-Gotland region diagnosed with breast cancer from 2001 to 2008[9, 13]. All individuals were identified through the Stockholm-Gotland Regional Breast Cancer Register. The Libro-1 cohort was established by inviting all women in Stockholm with breast cancer who were younger than age 80 years at diagnosis and diagnosed between 2001 and 2008 to participate. The overall response rate was 62%. In our study, we included women diagnosed with incident unilateral invasive breast cancer without any other previous cancer with the exception of non-melanoma skin cancer. We only included women post-menopausal at diagnosis with at least one pre-diagnostic mammogram of the contralateral breast, in the mediolateral oblique view, without a prior benign breast surgery, and where information on mode of detection was available. SDC, screen-detected breast cancer, was defined as a breast cancer diagnosis made after a positive screen finding but before the next visit or end of a normal screening interval. IC, interval breast cancer, was defined as a breast cancer diagnosis made after a negative screen but before the next regular visit or end of a normal screening interval. Symptomatic cases without previous screening were not included. During the study period, the overall screening participation rate in the Stockholm county was 70%, the recall rate was 3%, and the detection rate was 0.5% as described by Lind et al[14].

To validate our findings, we analyzed a second cohort of patients with post-menopausal breast cancer, the Cahres cohort[1]. It contained incident breast cancer cases diagnosed from October 1, 1993, to March 15,1995, and reported to any of the six Swedish Regional Cancer Registries. From this cohort, we were able to include 1,241 cases, of which 242 were IC and 999 were SDC as a validation population. The same inclusion and exclusion criteria were applied as for the primary study population. The reason for adding a validation cohort was to ensure that we had

not introduced overfitting through preliminary examination of alternative fluctuation measures in the primary study population.

Data collection

Data collection was performed similarly for the primary and the validation population. Information about BMI, HRT use, and other socio-demographic, anthropometric, hormonal, and lifestyle factors was obtained through questionnaires. Use of HRT was classified as 'current' or 'non-current' referring to the time-point of diagnosis. Data on age at menopause was only collected in the validation population.

We sought to retrieve all mammograms for the eligible women by using the Swedish national registration numbers given to all Swedish citizens at birth. We collected mammograms by contacting local mammography units as well as the national Swedish medical image repository in Vilhelmina, Sweden. In the study population there were 172 women with one mammogram, 178 women with two mammograms, and 1064 women with three or more mammograms. In total there were 5964 mammogram images. We did not have data on examination characteristics such as the brand of the mammography equipment or compression pressure applied by the mammography nurse.

All mammograms were analog film mammograms that were digitized using an Array 2905HD Laser Film Digitizer, which covers a range of 0 to 4.7 optical densities. The density resolution was set at 12-bit dynamic range. To avoid image acquisition bias related to suspected diagnosis we included only mammograms up until 60 days before the registered date of diagnosis. An automated method was used for PD measurement, which has previously been described in detail[15]. Briefly, the method attempts to mimic the gold standard PD measurement method Cumulus[16], which uses an automated thresholding procedure to obtain PD readings. For PD

measurement we used the mediolateral oblique view mammograms of the breast contralateral to the breast with the tumor. Using the contralateral image ensured that there could be no early tumor included in the PD measurements.

Statistical analysis

We used all available pre-diagnostic mammograms to estimate PD fluctuation by comparing the actual PD at each mammography with the PD predicted through modelling a smooth curve over time for each individual. The individual smooth PD curves were obtained by fitting a mixed effects regression model with PD as the outcome and age at mammography as the predictor. In all analyses, PD-based measures were square-root-transformed before modelling. We allowed PD to be a non-linear function of age by adding two cubic spline segments. Two segments were chosen since adding a third one resulted in non-significant beta coefficients. We allowed random effects for slope and intercept, with an unstructured covariance matrix. For each individual, at each mammography, we calculated the difference between the actual PD measure and the value predicted by the mixed effects model. The predicted values of the random effect depend upon the (unknown) covariance among the PD values, following the approach in section 8.6 in Fitzmaurice et al[17]. A single measure of PD fluctuation per individual was then calculated as the quadratic mean, or root-mean-square, of these differences; by using the quadratic mean rather than the arithmetic mean we ensured that deviations in opposite directions would not cancel each other out. Thus, the PD fluctuation measure is the average size, independent of the direction, of fluctuations away from the long-term PD trend.

We used logistic regression models to estimate the associations between IC/SDC status and PD fluctuation; first crude, then adjusted for PD at last pre-diagnostic examination, and finally a multiple adjusted model including PD fluctuation, pre-diagnostic PD, age at diagnosis, BMI and HRT use at diagnosis. We did not include family history of breast cancer, age at menarche or parity as covariates in our final model since they did not show any significant association with

IC/SDC status. Effect estimates are presented as odds ratios. For validation, we estimated the fully adjusted logistic regression model in an independent breast cancer cohort. Inherent to the mixed effects model used in the first stage of our analysis, the estimated fluctuation of a woman with less than three mammograms is largely based on data from the rest of the population. We examined how data from these women affected the estimated association between PD fluctuation through re-estimating the logistic regression model after exclusion of these individuals (those with less than three mammograms) in our primary study population. In order to illustrate the impact of having high or low PD fluctuation, we performed the fully adjusted logistic regression categorizing the continuous covariate PD fluctuation into quartiles. We then estimated the proportion of IC of the sum of IC and SDC breast cancer cases for each quartile of PD fluctuation. This was done holding the values of all other covariates fixed, reflecting a "typical" woman (age at diagnosis and BMI equal to the population mean and HRT status equal to 'non-current'). All statistical tests were two sided.

Our two-stage model implies that the uncertainty of the parameter estimates in the first stage model is not carried over to the estimation of the second stage model (i.e., predicted PD fluctuation is treated as a fixed variable at the second stage). Therefore, we also estimated a single-stage mixed effects model in the primary population, with PD as the outcome. In this model we allowed the variance of the random effect for the constant to vary according to IC/SDC status and included a fixed effect for IC/SDC status (as well as included fixed and random effects for age at mammography).

Informed consent and ethical approval

All participants provided written informed consent, and the study had approval from the ethical review board at Karolinska Institutet, Stockholm, Sweden.

RESULTS

In our primary study population we included 1414 women, 385 IC cases and 1029 SDC cases. The IC cases had a significantly lower age at diagnosis, a lower BMI, and were more frequently HRT users than were the SDC cases. The tumors of the IC cases were larger and had more often lymph node metastasis than the SDC cases [Table 1]. The average number of mammograms per woman was 4.3 for IC cases and 4.2 or SDC cases.

To illustrate the fact that a simple measure of variability (e.g., the standard deviation) would not differentiate between fluctuations and long-term trend we plotted the PD measurements over time for the women with the highest vs. the lowest of the raw measurements [Supplementary Figure 1]. Although there were some women in the high standard deviation group who had high fluctuations, and little long-term change, there were many women with high long-term change but relatively small fluctuations as well. This convinced us that it would be appropriate to use the proposed two-stage analysis, where the first stage involved estimating a mixed effects model [Supplementary Table 1]. As an illustration of how the individual long-term trend was positioned, we extracted observed PD measures and the corresponding model predicted smooth curve for two individuals, both with approximately 25 % PD at age 60 – one woman with large PD fluctuation and subsequent IC, and one woman with small PD fluctuation and subsequent SDC [Figure 1]. This figure illustrates that even though there is a long-term trend of declining PD, there is substantial fluctuation around that long-term trend.

We found that the PD fluctuation, the quadratic mean of deviations from the estimated long-term trend, was significantly higher for IC than for SDC (0.44 vs. 0.41, p=0.0309) [Table 2]. Supplementary Figure 2 shows the distributions of PD fluctuation for IC and SDC cases. Pre-diagnostic PD was significantly higher for IC than for SDC (25.5% vs. 20.3%, p<0.0001). There were no significant differences between IC and SDC cases regarding the following potential

confounders: total number of mammograms per person, average time between mammograms, mean age at mammography or time from first to last mammogram.

From fitting a logistic regression model with IC/SDC status as the outcome and (the model-based estimate of) PD fluctuation as the exposure we obtained an estimate of per-standard deviation OR of 1.14 (95% CI = 1.01 to 1.28) without adjustment [Table 3]. In the multiple adjusted model including last pre-diagnostic PD, age at diagnosis, BMI and HRT use at diagnosis as covariates, the OR was 1.17 (95% CI = 1.03 to 1.33) in the primary study population, and 1.19 (95% CI = 1.04 to 1.38) in the validation population. Exclusion of women with less than three mammograms, in the primary population, decreased the estimated OR in the multiple adjusted model slightly to 1.14 and widened the confidence interval (95% CI = 0.97 to 1.34). To examine the assumption of linearity underlying the OR per standard-deviation estimates, we added a quadratic term of the PD fluctuation variable. This term was not significantly associated with the outcome of the model, and we conclude that there is no strong evidence for such non-linearity. Combining the primary and the validation populations in a single logistic regression model and adding a categorical cohort identification covariate, we estimated the OR to the 1.16 (95% CI = 1.05 to 1.27). Adjusting for menopause transition during the time period of measurements changed the OR in the validation population to 1.17 (95% CI = 1.01 to 1.35), and the menopause transition variable was not significant in the model. In the primary study population data on age at menopause was not collected.

In Figure 2, we illustrate how the proportion of IC, out of the total of IC and SDC, differs between quartiles of PD Fluctuation in the primary study population. Setting all other covariates to the population average, we estimated that the proportion of IC was 19 percent in the lowest vs. 27 percent in the highest quartile of PD fluctuations.

The alternative single-stage modeling of PD over time including IC/SDC status as a covariate, showed that the additional variability in PD for individuals with IC (as opposed to SDC) status was significant (0.0228; 95% CI = 0.004 to 0.139), supporting the existence of differences in fluctuations between IC and SDC cases..

DISCUSSION

We have demonstrated that high longitudinal fluctuation in mammographic percent density of benign breast tissue is associated with an increased ratio of IC compared to SDC. The association was validated in an independent cohort, and was independent of last pre-diagnostic PD, age at diagnosis, BMI, and use of HRT. The proportion of interval cancer, out of interval and screen-detected together, increased from 19 to 27 percent between the lowest and highest quartile of mammographic density fluctuations.

In the first stage of our analysis, we applied mixed effects modeling of mammographic density as a function of age at mammography to estimate individual long-term trends. Unfortunately, such a complex approach is called for since simpler ones based on measuring variation without removing the underlying trend would not capture the relevant information for testing our hypothesis. Using splines when modeling density as a function of age at mammography allowed for a more rapid decrease in density around menopause in the population. In the second stage logistic regression we determined that there was an association between density fluctuations and the ratio of interval vs. screen-detected cancer also after taking age at diagnosis, BMI and use of HRT into account. In the validation population we were able to determine that this association did not materially change after taking into consideration whether a woman had a menopause transition during the period of sequential mammograms. Exclusion of women with less than 3 mammograms weakened the identified association slightly, and decreased the precision of the analysis.

We hypothesize that large fluctuations of mammographic density can result in reduced radiological screening detectability and consequently in an increased ratio of IC compared to SDC. In addition, large fluctuation might also be a marker of an intramammary environment promoting faster-growing tumors. Weekly fluctuations in breast tissue, in both pre- and postmenopausal women, have been demonstrated in a previous MRI-based study [18]. There is some evidence from a mammography study that tissue fluctuations might be correlated with phases in the menstrual cycle [19]. There have not been any studies explaining the basis for potential tissue fluctuations among post-menopausal women. The total density fluctuation observed in this study would be a combination of true tissue fluctuations and artificial fluctuations caused by differences in examination technique and mammography nurse practices between sequential mammograms. Our original hypothesis was based on fluctuations representing variations between sequential mammograms making it more difficult for the screening radiologist to discern a subtle malignant change. Therefore, the total fluctuation between images should be the most relevant measure. However, in future research, it would be of interest to control for differences in examination characteristics between examinations. That would enable us to better understand whether fluctuations may be related to some biological characteristics that are associated with faster-growing tumors or less visible histological subtypes.

One strength of our study is that we were able to collect a large number of pre-diagnostic mammograms for most of the women. Another strength is that we had extensive information on established IC determinants, with a low rate of missing information, allowing appropriate adjustments for confounders. A final strength is that we were able to validate our main finding in an independent breast cancer cohort. A limitation of the study is that is based on a case-only cohort, from which conclusions for changing screening policy cannot be drawn. Another limitation is that the age of menopause was only known in the validation population. A potential modeling limitation of the two-stage approach in our study is that the uncertainty in estimating

PD fluctuation at the first stage is not carried over to the second stage. Nevertheless, both this model and the single-stage model resulted in the same conclusion regarding a difference in PD fluctuation between IC and SDC cases.

In conclusion, based on a case-only cohort, large longitudinal fluctuation in mammographic percent density increases the ratio of interval compared to screen-detected breast cancer. Whether this is entirely related to a reduced mammographic detectability or also to a biological phenotype promoting faster tumor growth remains to be elucidated. The association between interval cancer and density fluctuations should be further assessed in a screening cohort including healthy women before considered as a potential marker of elevated risk of interval cancer and thus potentially applied to direct additional screening resources.

Ethics, consent and permissions

All participants provided written informed consent to participate, and the studies had approval from the ethics committee at Karolinska Institutet, Stockholm, Sweden (Dnr 2009/254-31/4).

REFERENCES

1. Eriksson L, Czene K, Rosenberg LU, Törnberg S, Humphreys K, Hall P. Mammographic density and survival in interval breast cancers. *Breast cancer research : BCR* 2013;**15**: R48.

2. Kirsh VA, Chiarelli AM, Edwards SA, O'Malley FP, Shumak RS, Yaffe MJ, Boyd NF. Tumor characteristics associated with mammographic detection of breast cancer in the Ontario breast screening program. *Journal of the National Cancer Institute* 2011;**103**: 942-50.

3. Gilliland FD, Joste N, Stauber PM, Hunt WC, Rosenberg R, Redlich G, Key CR. Biologic characteristics of interval and screen-detected breast cancers. *Journal of the National Cancer Institute* 2000;**92**: 743-9.

4. Porter PL, El-Bastawissi AY, Mandelson MT, Lin MG, Khalid N, Watney EA, Cousens L, White D, Taplin S, White E. Breast tumor characteristics as predictors of mammographic detection: comparison of interval-and screen-detected cancers. *Journal of the National Cancer Institute* 1999;**91**: 2020-8.

5. Domingo L, Blanch J, Servitja S, Corominas JM, Murta-Nascimento C, Rueda A, Redondo M, Castells X, Sala M. Aggressiveness features and outcomes of true interval cancers: comparison between screen-detected and symptom-detected cancers. *European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP)* 2013;**22**: 21-8.

6. Domingo L, Sala M, Servitja S, Corominas JM, Ferrer F, Martinez J, Macia F, Quintana MJ, Albanell J, Castells X. Phenotypic characterization and risk factors for interval breast cancers in a population-based breast cancer screening program in Barcelona, Spain. *Cancer causes & control : CCC* 2010;**21**: 1155-64.

7. Andersen SB, Tornberg S, Lynge E, Von Euler-Chelpin M, Njor SH. A simple way to measure the burden of interval cancers in breast cancer screening. *BMC cancer* 2014;**14**: 782.

8. Chiarelli AM, Kirsh VA, Klar NS, Shumak R, Jong R, Fishell E, Yaffe MJ, Boyd NF. Influence of patterns of hormone replacement therapy use and mammographic density on breast cancer detection. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2006;**15**: 1856-62.

9. Holm J, Humphreys K, Li J, Ploner A, Cheddad A, Eriksson M, Tornberg S, Hall P, Czene K. Risk factors and tumor characteristics of interval cancers by mammographic density. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015;**33**: 1030-7.

10. van Gils CH, Otten JD, Verbeek AL, Hendriks JH. Mammographic breast density and risk of breast cancer: masking bias or causality? *European journal of epidemiology* 1998;**14**: 315-20.

11. Boyd N, Guo H, Martin L, Sun L, Stone J, Fishell E, Jong R, Hislop G, Chiarelli A, Minkin S, Yaffe M. Mammographic density and the risk and detection of breast cancer. *The New England journal of medicine* 2007;**356**: 227 - 36.

12. Maskarinec G, Pagano I, Lurie G, Kolonel LN. A longitudinal investigation of mammographic density: the multiethnic cohort. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2006;**15**: 732-9.

13. Cheddad A, Czene K, Shepherd JA, Li J, Hall P, Humphreys K. Enhancement of mammographic density measures in breast cancer risk prediction. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2014;**23**: 1314-23.

14. Lind H, Svane G, Kemetli L, Tornberg S. Breast Cancer Screening Program in Stockholm County, Sweden - Aspects of Organization and Quality Assurance. *Breast care (Basel, Switzerland)* 2010;**5**: 353-7.

15. Li J, Szekely L, Eriksson L, Heddson B, Sundbom A, Czene K, Hall P, Humphreys K. High-throughput mammographic-density measurement: a tool for risk prediction of breast cancer. *Breast cancer research : BCR* 2012;**14**: R114.

16. Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ. The quantitative analysis of mammographic densities. *Physics in medicine and biology* 1994;**39**: 1629-38.

17. Fitzmaurice GM, Laird NM, Ware JH. *Applied longitudinal analysis*ed., vol. 998: John Wiley & Sons, 2012.

18. Chan S, Su MY, Lei FJ, Wu JP, Lin M, Nalcioglu O, Feig SA, Chen JH. Menstrual cycle-related fluctuations in breast density measured by using three-dimensional MR imaging. *Radiology* 2011;**261**: 744-51.

19. Morrow M, Chatterton RT, Jr., Rademaker AW, Hou N, Jordan VC, Hendrick RE, Khan SA. A prospective study of variability in mammographic density during the menstrual cycle. *Breast cancer research and treatment* 2010;**121**: 565-74.

Table 1 Patient characteristics

	Study population (n=1,414)			
	IC (n=385) n (%) or mean	SDC (n=1,029) n (%) or mean	p value	Missing data
Age at diagnosis	61.2	61.7	0.0417	0%
BMI	24.9	25.8	0.0004	1.3%
Oral contraceptive use			0.863	1.0%
No	290 (77%)	779 (76%)		
Yes	89 (23%)	245 (24%)		
HRT use at diagnosis			<0.001	2.4%
No	287 (76%)	852 (85%)		
Yes	89 (24%)	152 (15%)		
First-degree relative with breast cancer			0.092	6.1%
No	278 (77%)	781 (81%)		
Yes	85 (23%)	186 (19%)		
Tumor size, mm	19.8	15.6	<0.0001	1.3%
Lymph node metastasis			<0.0001	7.8%
Negative	332 (87%)	972 (95%)		
Positive	50 (13%)	56 (5%)		

IC = Interval breast cancer
SDC = Screen-detected breast cancer
p-values for difference between the IC and SDC group were calculated by two-sided t-test for continuous variables; and by chi square tests for categorical variables.

Table 2 Mammographic characteristics

	Study population (n=1,414)				
	IC (n=385)		SDC (n=1032)		p value
	mean	(SD)	mean	(SD)	
PD fluctuation, RMS	0.44	(0.22)	0.41	(0.22)	0.0309
PD last pre-diagnostic, percent	26	(16)	20	(13)	<0.0001
Time between mammogram rounds, years	1.64	(1.14)	1.62	(1.05)	0.6939
Age at mammography, years	55.6	(4.80)	55.4	(4.17)	0.3348
Time from first to last mammogram, years	7.74	(5.53)	7.47	(5.20)	0.4054
Number of mammograms	*n*	(proportion)	*n*	(proportion)	0.681
1	47	(12%)	125	(12%)	
2	50	(13%)	128	(12%)	
3 or more	288	(75%)	776	(75%)	

IC = Interval breast cancer
SDC = Screen-detected breast cancer
PD fluctuation = Measure of deviations from the long-term trend of PD
RMS = Root-mean-square
SD=standard deviation
p-values for difference between the IC and SDC group were calculated by two-sided t-test
In all tests, PD-based measures were square-root-transformed

Table 3 Associations between PD fluctuation and IC vs SDC status

	Primary cohort (Libro-1)	Validation cohort (Cahres)
Adjustments	*Odds ratio (95% C.I.)*	*Odds ratio (95% C.I.)*
Crude	1.14 (1.01 to 1.28)	1.22 (1.06 to 1.40)
PD adjusted	1.14 (1.02 to 1.29)	1.18 (1.03 to 1.36)
Multiple adjusted	1.17 (1.03 to 1.33)	1.19 (1.04 to 1.38)

PD fluctuation = RMS measure of deviation, including all prediagnostic mammograms, compared to the individual long-term trend of PD
Odds Ratios were estimated by logistic regression with IC vs SDC status as outcome
Covariates in the 'Multiple adjusted' model are PD, age at diagnosis, BMI and HRT use
IC = Interval breast cancer
SDC = Screen-detected breast cancer
In all tests PD-based covariates were square-root-transformed

Supplementary Table 1. Parameter estimates from the (stage 1) mixed effects model in the primary population.

Mixed effects model

$sqrt(PD) = \beta_0 + \beta_1 \times age\_spline1 + \beta_2 \times age\_spline2 + u_{i0} + u_{i1} \times age\_spline1 + u_{i2} \times age\_spline2 + e_{ij}$

Parameter estimates in primary study population

Fixed effects parameters

β_0 : 6.58 (95% CI: 6.43 to 6.74)

β_1 : -0.00979 (95% CI: -0.0107 to -0.0089)

β_2 : 0.00166 (95% CI: 0.00046 to 0.00286)

Random effects parameters

$\sigma^2(u_{i0})$: 2.101 (95% CI: 1.673 to 2.640)

$\sigma^2(u_{i1})$: 0.0000447 (95% CI: 0.0000313 to 0.0000638)

$\sigma^2(u_{i2})$: 0.0000371 (95% CI: 0.0000174 to 0.0000791)

$\sigma(u_{i0}, u_{i1})$: -0.00520 (95% CI: -0.00769 to -0.002712)

$\sigma(u_{i0}, u_{i2})$: -0.000976 (95% CI: -0.00200 to 0.00396)

$\sigma(u_{i1}, u_{i2})$: -0.000027 (95% CI: -0.000046 to -0.000008)

Residual

$\sigma^2(e_{ij})$: 0.378 (95% CI: 0.360 to 0.396)

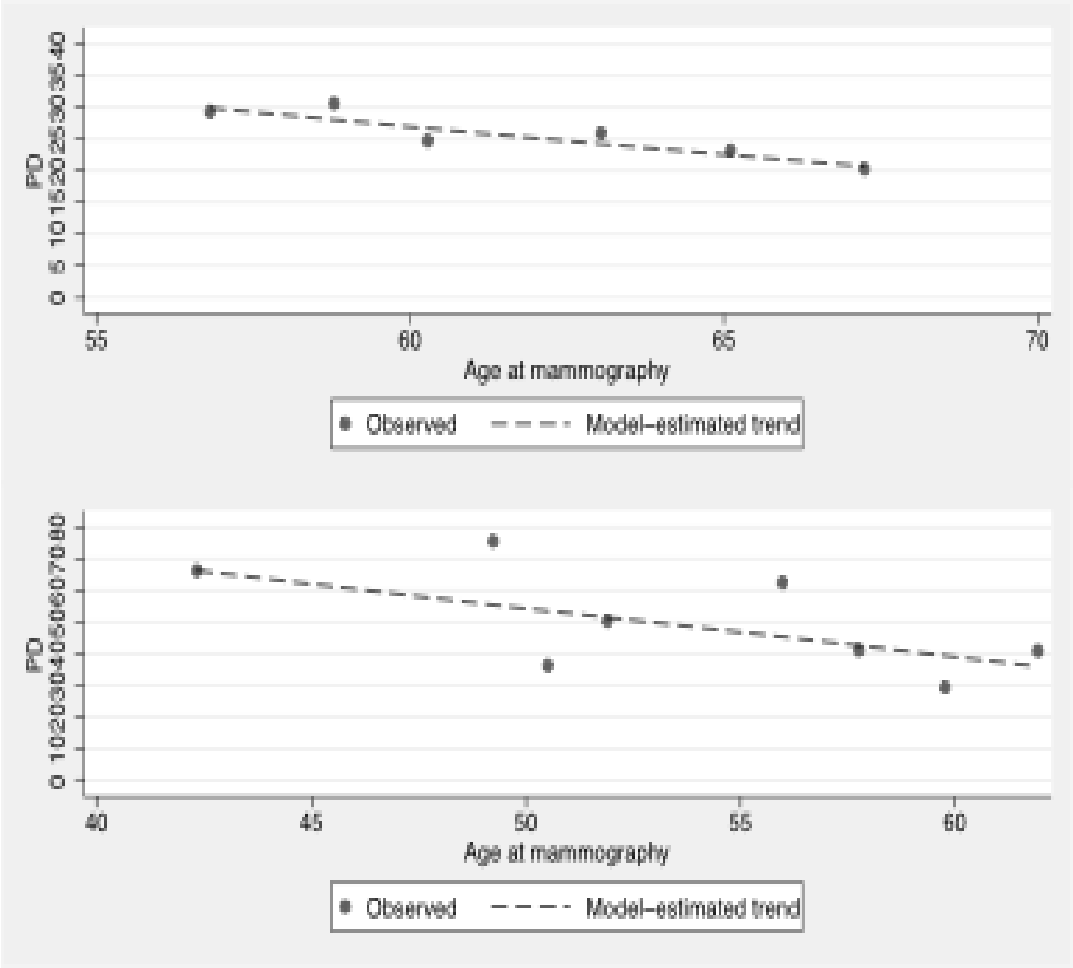

PD
0 5 10 15 20 25 30 35 40
55 60 65 70
Age at mammography
Observed
Model–estimated trend
PD
0 10 20 30 40 50 60 70 80
40 45 50 55 60
Age at mammography
Observed
Model–estimated trend

FIGURE 1

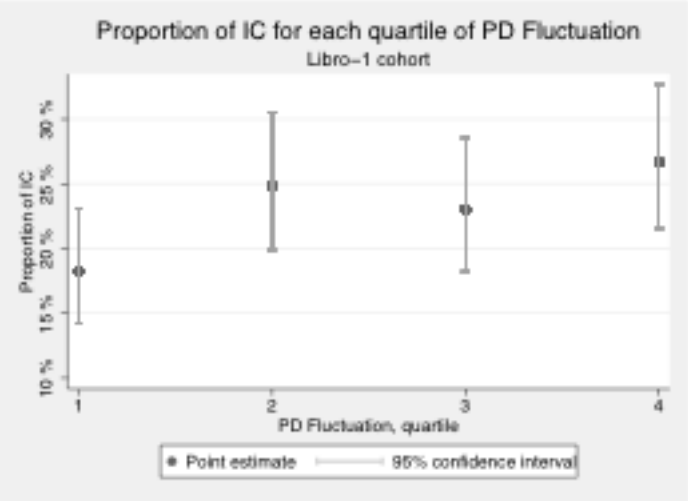
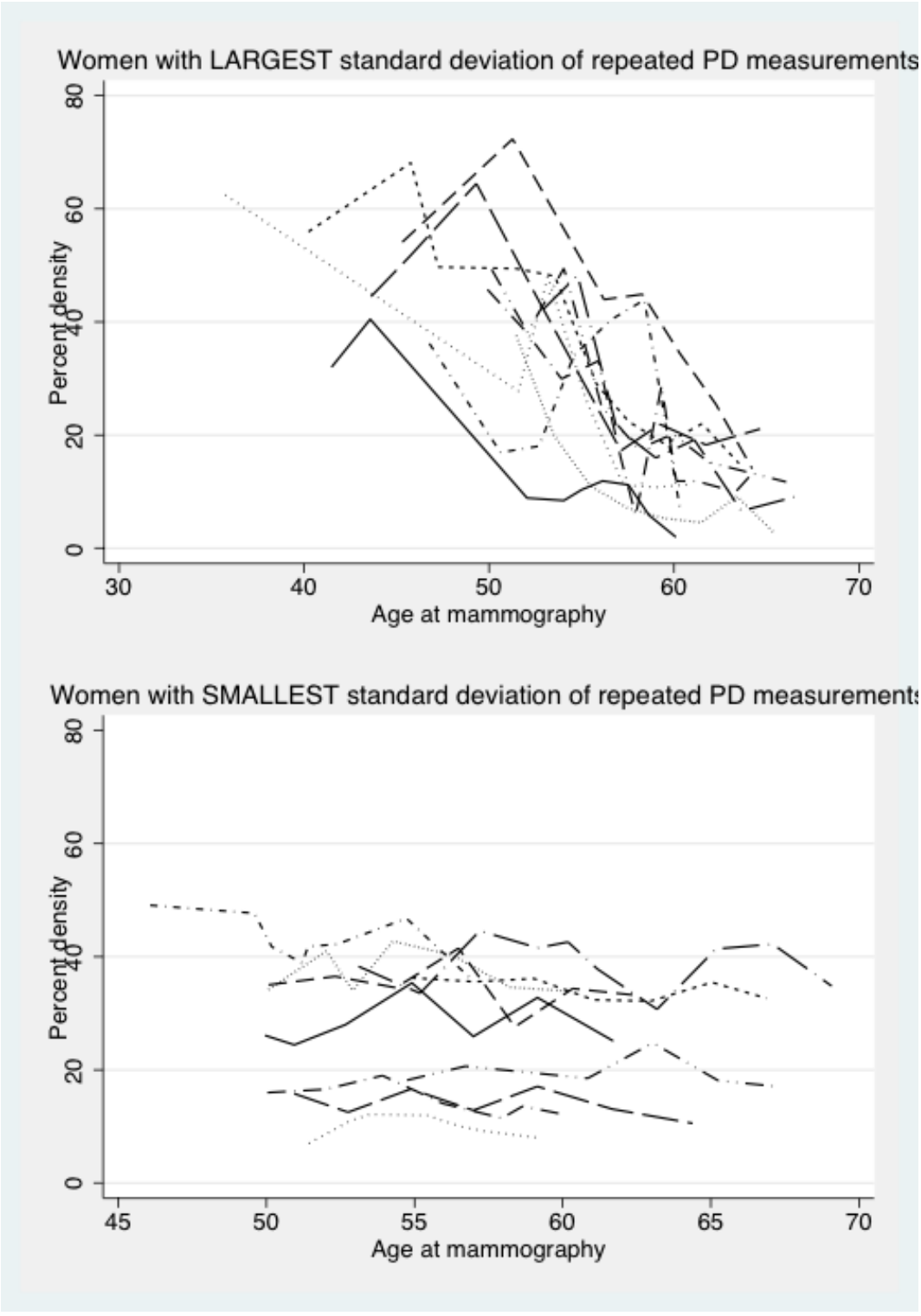

Proportion of IC for each quartile of PD Fluctuation
Libro-1 cohort
Proportion of IC
10 %
15 %
20 %
25 %
30 %
1
2
3
4
PD Fluctuation, quartile
Point estimate
95% confidence interval

FIGURE 2



SUPPLEMENTARY FIGURE 1