Molecular subtypes of breast cancer: long-term incidence trends and prognostic differences.

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Molecular subtypes of breast cancer: long-term incidence trends and prognostic differences.

Background: Secular trends in incidence and prognosis of molecular breast cancer subtypes are poorly described. We studied long-term trends in a population of Norwegian women born 1886-1977.

Methods: A total of 52 949 women were followed for breast cancer incidence, and 1423 tumours were reclassified into molecular subtypes using immunohistochemistry and *in situ* hybridization. We compared incidence rates among women born 1886-1928 and 1929-1977, estimated age-specific incidence rate ratios (IRRs), and performed multiple imputations to account for unknown subtype. Prognosis was compared for women diagnosed before 1995 and in 1995 or later, estimating cumulative risk of death and hazard ratios (HR).

Results: Between 50-69 years of age, incidence rates of Luminal A and Luminal B (Human epidermal growth factor receptor (HER2)-) were higher among women born in 1929 or later, compared to before 1929 (IRRs 50-54 years, after imputations: 3.5, 95% confidence interval (CI) 1.8-6.9 and 2.5, 95% CI 1.2-5.2, respectively), with no clear differences for other subtypes. Rates of death were lower in women diagnosed in 1995 or later, compared to before 1995, for Luminal A (HR 0.4, 95% CI 0.3-0.5), Luminal B (HER2-) (HR 0.5, 95% CI 0.3-0.7), and Basal phenotype (HR 0.4, 95% CI 0.2-0.9).

Conclusion: We found a strong secular incidence increase restricted to Luminal A and Luminal B (HER2-) subtypes, combined with a markedly improved prognosis for these subtypes and for the Basal phenotype.

Impact: This study documents a clear secular increase in incidence and a concomitant improved prognosis for specific molecular breast cancer subtypes.

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Molecular subtypes of breast cancer: long-term incidence trends and prognostic differences.

Introduction

Breast cancer incidence rates have gradually increased in Norway since the 1950s (1, 2), with a markedly stronger increase starting in the early 1990s. Breast cancer mortality remained stable from the 1950s until around 1995, since then there has been a clear and consistent decline (1). Similar changes in incidence and mortality have been observed in most developed countries (3-6). However, long-term secular trends in incidence and prognosis of molecular subtypes of breast cancer are poorly documented.

The heterogeneous nature of breast cancer that is observed both clinically and histopathologically, is also apparent in gene expression patterns (7, 8). Using immunohistochemistry (IHC) and *in situ* hybridization (ISH) as surrogates for gene expression analysis, archival tumour tissue can be reclassified into molecular subtypes (7-12). We used IHC and ISH to reclassify incident tumours into six subtypes: Luminal A [oestrogen receptor (ER) and/or progesterone receptor (PR)+, human epidermal growth factor receptor 2 (HER2)-, Ki67<15%], Luminal B (HER2-) [ER and/or PR+, HER2-, Ki67≥15%], Luminal B (HER2+) [ER and/or PR+, HER2+], HER2 type [ER-, PR-, HER2+], 5 negative phenotype [ER-, PR-, HER2-, Cytokeratin 5 (CK5)-, and epidermal growth factor receptor (EGFR)-], and Basal phenotype [ER-, PR-, HER2-, CK5+ and/or EGFR+].

Our main aim was to study long-term trends in incidence of different molecular breast cancer subtypes in a population of Norwegian women born between 1886 and 1977. Our second aim was to study the prognosis of molecular breast cancer subtypes diagnosed among these women.

Materials and methods

This follow-up study comprises women from two population-based surveys conducted in Nord-Trøndelag County, Norway. Information on incident breast cancer was obtained from the Cancer Registry of Norway, date of death and/or emigration from Statistics Norway, and causes of death from the Norwegian Cause of Death Registry. Pathology reports and formalin-fixed, paraffin-embedded (FFPE) tissue from the first primary tumour were retrieved from the Department of Pathology and Medical Genetics at St. Olav's Hospital, Trondheim University Hospital, Norway.

Cohort 1. The first survey was conducted between 1956 and 1959, as part of a larger study that also included two other counties (13). We studied women from Nord-Trøndelag County, comprising a total of 25 727 women born between 1886 and 1928 who were followed for breast cancer occurrence from January 1st, 1961 until December 31st, 2008. Follow-up was facilitated by the introduction of the unique 11-digit identity number of all Norwegian citizens in 1961. In total, 1379 incident cases were diagnosed during follow-up, and 909 of these tumours were previously subtyped by our group (11). Some tumours were diagnosed at other hospitals, in particular in the 1960s and 1970s, and tumour tissue from these cases was not available for this study. After diagnosis, all patients were followed until death from breast cancer or death from other causes, or until December 31st, 2010.

Cohort 2. The second survey was conducted between 1995 and 1997. In this study, all women in Nord-Trøndelag County aged 20 years or older were invited to participate in the second wave of the HUNT Study in Nord-Trøndelag (14). A total of 34 221 women born between 1897 and 1977 participated. From attendance until December 31st, 2009, 728 women were diagnosed with breast cancer. Of these, 157 were already included in Cohort 1. Of the remaining tumours, 57 were unavailable for subtyping, resulting in a total of 514 tumours from Cohort 2 that were subtyped in the present study (Figure 1). After diagnosis, these

patients were followed until death from breast cancer or death from other causes, or until December 31st, 2013.

In the present study, we merged data from the two cohorts (Figure 1). In accordance with the requirements and conditions of the ethical approval of the study, patient identity was known to us for breast cancer cases but not for the underlying populations. Since there was some overlap in birth year between Cohort 1 and 2, we restricted Cohort 2 to women born after 1928 (n=27 222) to avoid duplicate observations in the incidence analyses. In the restricted cohort, there were 529 incident breast cancers, including 480 of the 514 cases that could be subtyped. In the analysis of incidence rates, we therefore used data from a total of 1908 incident breast cancers that occurred among 52 949 women; 1379 (909 subtyped cases) from Cohort 1, and 529 (480 subtyped cases) from Cohort 2.

In the analyses of prognosis for different breast cancer subtypes, we included all 514 cases from Cohort 2 and the 909 cases from Cohort 1, yielding a total of 1423 subtyped cases.

Specimen characteristics

New 4-μm thick sections from representative paraffin blocks were stained with haematoxylin–erythrosine–saffron (HES), reviewed by two pathologists independently, and classified into histopathological type and grade (15, 16). Any discrepancies were discussed, and consensus reached. Tumour size was measured on the glass slide, and correlated to information in the pathology report. In cases with multifocal tumours, the largest tumour was selected.

Tissue microarrays (TMA) were constructed using the Tissue Arrayer MiniCore® 3 with TMA Designer2 software (Alphelys, 78370 Plaisir, France). Three tissue cores (1-mm in diameter) from the tumour periphery were inserted into TMA recipient blocks, and 4-μm thick sections were cut and mounted on Superfrost+ glass slides, dried at 37°C overnight, and

stored in the freezer at -20°C. For IHC, slides were heated to 60°C for 2 hours, and pre-treated in a PT Link, Pre-Treatment Module for Tissue Specimens (Dako Denmark A/S, 2600 Glostrup, DK) with buffer (Low pH Target Retrieval Solution K8005 for Ki67, High pH Target Retrieval Solution K8004 for all other markers) at 97°C for 20 minutes.

Slides were stained with HES, and immunostaining for ER (Clone SP1, concentration 33 mg/mL, dilution 1:100, Cell Marque, Rocklin, United States), PR (Clone 16, concentration 360 mg/L, dilution 1:400, NovoCastra Laboratories, Newcastle Upon Tyne, UK), HER2 (Clone CB11, concentration 3.9 g/L, dilution 1:640, Novocastra), the proliferation marker Ki67 (Clone MIB1, concentration 35 mg/L, dilution 1:100, Dako Denmark A/S, Glostrup, Denmark), and basal markers CK5 (Clone XM26, concentration 50 mg/l, dilution 1:100, Novocastra) and EGFR (Clone 2-18C9, concentration ready to use, no dilution, Dako) was done in a DakoCytomationAutostainer Plus (Dako). Dako REAL™EnVision™ Detection System with Peroxidase/DAB+, Rabbit/Mouse, code K5007, was used for visualization for all markers except EGFR. EGFR was immunostained and visualized with EGFR pharmDX™ kit, code K1494 (Dako). Negative controls were included in all staining runs.

Chromogenic ISH (Cohort 1) and fluorescence ISH (Cohort 2) were used to demonstrate the *HER2* gene and chromosome 17 centromere. The dual-colour probe kit HER2 CISH pharmDx™ Kit, code 109 (Dako) was used for CISH (11), and HER2 IQFISH DAKO pharmDX Kit K 5731 (Dako) was used for FISH. Pre-treatment was done with pepsin solution at 37˚C for 25 minutes for both CISH and FISH.

Scoring and reporting

Slides were scanned using Ariol™ SL-50 3.3 Scan system (Genetix Europe Ltd., Gateshead, UK). IHC markers were assessed by two researchers independently. Discrepant results were discussed, and consensus reached.

HER2 status was assessed with a bright-field microscope (Nikon Eclipse 80*i*) (Cohort 1) and a fluorescence microscope (Nikon Eclipse 90i) with Cytovision software version 3.7 (Applied Imaging International Ltd., Newcastle-upon-Tyne, UK) (Cohort 2).

Classification of markers

ER and PR were positive when ≥1% of tumour nuclei showed positive staining, irrespective of staining intensity (17). Ki67 was counted in 500 tumour cells (hotspots), and considered high when ≥15% of nuclei were positive, irrespective of staining intensity (10, 18-20). Membranous staining for HER2 was scored from 0 to +3, (0/+1 negative; +2 borderline/equivocal; +3 positive) (21). *HER2* amplification was defined as a gene to chromosome ratio ≥2. At least 20 non-overlapping, well-preserved tumour cell nuclei with signals for both *HER2* and chromosome 17 centromere were assessed. Tumours with unsuccessful ISH, but IHC +3, were considered positive.

For CK5 and EGFR, a staining index was calculated by multiplying the proportion of positive staining cells (1 (<10 %); 2 (10-50 %); 3 (>50 %)) by staining pattern/intensity. Staining intensity for CK5 was defined as 0 (no staining); 1 (weak); 2 (moderate) and 3 (strong). For EGFR, membranous staining was 0 (no staining); 1 (faint, incomplete staining); 2 (moderate intensity, circumferential staining); 3 (strong intensity, circumferential staining), according to Dako PharmDX kit guidelines. A staining index of 0-1 was classified as negative, 2-9 as positive. The REMARK recommendations for reporting tumour marker studies were followed (22).

Classification of tumours

Tumours were classified into the following six molecular subtypes: Luminal A, Luminal B (HER2-), Luminal B (HER2+), HER2 type, 5 negative phenotype and Basal phenotype, based on IHC and ISH results, as previously described (11).

To allow comparison with previous studies (23-25), tumours were also classified into four subtypes according to hormone receptor- and HER2 status: ER+ and/or PR+, HER2-; ER+ and/or PR+, HER2+; ER- and PR-, HER2+, and ER- and PR-, HER2-. The results are presented as supplementary material.

Statistical analyses

During follow-up for breast cancer occurrence, censoring was done at time of death or emigration. Incidence rates were estimated separately for women born before 1929 and women born in 1929 or later. Age-specific rates were calculated to account for differences in age at baseline, and variations in age at diagnosis between subtypes. Estimates of incidence rates were plotted according to birth year and age for all incident cancers combined, and for each subtype separately. Poisson regression was used to compare incidence rates between women born before 1929 and women born in 1929 or later. The data allowed comparison of incidence rates in the age range 50-69 years. In the comparisons of Luminal A and Luminal B (HER2-), we had sufficient statistical power to use 5-year categories of age within that age-range, estimated as incidence rate ratios (IRRs) with 95% confidence intervals (CI). For the remaining subtypes, statistical power was limited and we used 10-year categories in the incidence comparisons.

For some cases, tumour tissue was unavailable, or the tumours could not be subtyped for other reasons. Thus, tumours from 34% of cases born before 1929, and 9% of cases born in 1929 or later could not be subtyped. Consequently, the observed subtype-specific incidence rates would underestimate the true rates, and underestimation would be greater for women

born before 1929, because their tumour subtype was more likely to be unknown. To compensate for this, we performed multiple imputations to predict the molecular subtype of these tumours (24, 26), assuming samples were missing at random (27). The imputation model included all information available: age (5-year categories) and calendar year at diagnosis (continuous), stage (I, II, III, IV, unknown) and extent of disease (disease localized to the breast, local invasion, regional lymph nodes, distant lymph nodes or organ metastases, unknown) as reported by the Cancer Registry of Norway, year of birth (5-year categories), observation time after diagnosis (log-transformed, continuous) and survival status (alive, death from breast cancer, death from other causes). Excluding each of the following variables in turn; stage, extent of disease or survival time, had no major influence on the imputed rates, nor did changing the categorization of continuous variables. Descriptive statistics for the information used in the imputation models are available in Supplementary Table 1. Incidence rates with 95% CIs were calculated based on 50 imputed data sets according to birth year and 5-year age categories.

In analyses of prognosis, we distinguished between women diagnosed before 1995 and women diagnosed in 1995 or later, to approximate the gradual implementation of adjuvant treatment (including effective chemotherapy, anti-hormonal treatment and trastuzumab) in Norway (28). For each subtype, we calculated cumulative incidence of death from breast cancer at 5 and 15 years after diagnosis, treating deaths from other causes as competing events. Gray's test was used to test equality between cumulative incidence curves.

We used Cox proportional hazards models to compare the rate of death within each diagnostic period according to molecular subtype, and to compare the rate of death for each subtype between diagnostic periods. In the latter analysis, estimations were made for the first 5 and 15 years after diagnosis, and for the entire follow-up period. We estimated hazard ratios (HRs) with 95% CIs from the month of diagnosis until death, with censoring at time of death

from other causes, and with adjustments for age, stage and histopathological grade at diagnosis. No clear violations of proportionality were found in log-minus-log plots. Stata version 13.1 (Stata Corp., College Station, TX, USA) was used for statistical analyses.

Ethical approval

The study was approved by the Regional Committee for Medical and Health Sciences Research Ethics (REK, Midt-Norge, Norway ref. nr: 836/2009).

Results

Age-specific incidence rates according to year of birth

Mean age at baseline was 51.0 years for women born before 1929, and 43.4 years for women born in 1929 or later. Mean follow-up times in the two groups of women were 29.7 and 13.1 years, respectively.

Between 50 and 69 years of age, total breast cancer incidence was higher for women born in 1929 or later, compared to women born before 1929 (Table 1, Supplementary Figure 1). In subtype-specific analyses, incidence rates of Luminal A and Luminal B (HER2-) were consistently higher in women born in 1929 or later (Table 1, Figure 2). The higher incidence was particularly evident in the age group 50-54 years (IRR 7.7, 95% CI 3.4-17.4 and IRR 5.9, 95% CI 2.4-14.5, respectively) and weaker in the 65-69 year age group (IRR 2.3, 95% CI 1.6-3.5 for Luminal A and IRR 1.2, 95% CI 0.6-2.3 for Luminal B (HER2-)). Although the incidence rates for Luminal B (HER2+) and non-luminal subtypes were also higher for women born in 1929 or later, the differences were much less pronounced and varied considerably between age groups.

After imputation for unknown subtype, the observed relative rates (IRR) for Luminal A and Luminal B (HER-) were strongly attenuated (Table 1). Thus, the IRR for Luminal A breast cancer in the age group 50-54 years was reduced from 7.7 to 3.5 (95% CI 1.8-6.9), and for Luminal B (HER2-), there was a corresponding reduction in IRR from 5.9 to 2.5 (95% CI 1.2-5.2) after imputation. The IRRs for Luminal B (HER2+) and the non-luminal subtypes were almost fully attenuated after imputation (Table 1).

Incidence analysis based on observed data for the four subtypes determined by ER, PR, and HER2 status showed a marked incidence increase for the ER+, PR+, HER2- subtype, with an IRR of 6.9 (95% CI 3.8-12.6) for the age group 50-54 years. After imputation, the IRR was attenuated to 3.1 (95% CI 1.9-5.1). The results of these analyses are reported in detail in Supplementary Table 2 and Supplementary Figure 2.

Prognosis according to molecular subtype and year of diagnosis

Mean follow-up after diagnosis was 9.8 years for patients diagnosed before 1995, and 7.9 years for patients diagnosed in 1995 or later. Women diagnosed in 1995 or later were on average younger, and their tumours were more often Luminal A and of lower grade. Furthermore, tumours diagnosed in 1995 or later were generally smaller compared to those diagnosed before 1995 (Table 2). However, information on tumour size was frequently missing or insufficiently described in the pathology reports from the first diagnostic period.

In both diagnostic periods, Luminal A had the best prognosis, and HER2 type had the poorest (Table 3, Figure 3). Although the absolute risks of death for each subtype differed between diagnostic periods, the patterns of risk between subtypes remained roughly similar.

The cumulative risk of death from Luminal A breast cancer was 37 % (95% CI 32-44%) after 15 years of follow-up for women diagnosed before 1995 (Table 3, Figure 3), and 13% (95% CI 9-17 %) in women diagnosed in 1995 or later, indicating a strong decline in

case fatality from the first to the second diagnostic period. The corresponding cumulative risk of death for women diagnosed with HER2 type was 57% (95% CI 44-71%) and 42% (95% CI 28-60%).

We used Cox regression analysis to compare rates of death between subtypes in each diagnostic period, and found that among women diagnosed before 1995, the rate of death from HER2 type was more than twice as high (age-adjusted HR 2.3, 95% CI 1.5-3.5) as for Luminal A. The corresponding HR for women diagnosed with HER2 type in 1995 or later was much higher (age-adjusted HR 5.1, 95% CI 2.8-9.3). Adjusting for histopathological grade or stage of disease at diagnosis did not substantially influence these results.

We also used Cox regression analysis to compare rates of death for each subtype between diagnostic periods (Table 3). Generally, rates of death were lower for women diagnosed in 1995 or later, although precision was low for the less common subtypes. Thus, for the entire follow-up period, the rate of death was 60% lower for Luminal A (age-adjusted HR 0.4, 95% CI 0.3-0.5), 50% lower for Luminal B (HER2-) (age-adjusted HR 0.5, 95% CI 0.3-0.7), and 60% lower for Basal phenotype (age-adjusted HR 0.4, 95% CI 0.2-0.9). Changes between diagnostic periods for the other subtypes were less apparent (Table 3). The results remained similar when analyses were restricted to the first 5 and 15 years after diagnosis, with clear improvements in survival between diagnostic periods for Luminal A, Luminal B (HER2-) and Basal phenotype (Supplementary Table 3). Analyses based on the four subtypes determined by ER, PR, and HER2 status showed that both before and after 1995, the ER+, PR+, HER2- subtype had the best prognosis (Supplementary Table 4, and Supplementary Figure 3). Comparing prognosis between diagnostic periods, clear improvements were seen for the ER+, PR+, HER2- subtype (HR 0.4 (95% CI 0.3-0.5)), and for the triple negative (ER-, PR-, HER2-) subtype (HR 0.5 (95% CI 0.3-0.9)) (Supplementary Tables 4 and 5).

Discussion

This large population-based study of women born between 1886 and 1977 shows that for women aged 50-69 years, the incidence of breast cancer was higher among those born in 1929 or later, compared to women born before 1929. This was primarily due to a much higher incidence of the low-proliferative Luminal A tumours, but also to some extent for Luminal B (HER2-) tumours. The prognosis was generally better for women diagnosed in 1995 or later, compared to before 1995, but clear improvements in prognosis were seen for Luminal A, Luminal B (HER2-), and the Basal phenotype. Luminal A had the best prognosis and HER2 type had the poorest in both diagnostic periods.

The participants came from a single county in Norway, which is predominantly rural and ethnically homogeneous, with little migration (14). This increases the comparability over time within the study population. Incident tumours were reclassified into molecular subtypes and included in analyses of incidence and long-term prognosis, using reliable end-point data from national registries.

Molecular subtyping was performed in the same laboratory, using the same antibodies for IHC in all tumours. This ensured that the observed incidence differences were not caused by different antibody sensitivities or cut-off levels. Subtyping of tumours was done according to the same algorithm. Tumour tissue covered a diagnostic time span of several decades, and although preanalytical conditions may have varied, valuable information can be drawn from archival tissue blocks (29).

Most breast cancers are hormone receptor positive (luminal) and HER2 negative. Our results are in agreement with previous studies showing that HER2 negative luminal tumours are more common among older, postmenopausal women (30, 31), and that non-luminal subtypes are more common in younger women (30, 31).

Increased incidence of breast cancer over time has been reported by others (3, 4, 32), and ER positive tumours may account for most of this increase (3, 32, 33). It has been suggested that mammography screening favours detection of HER2 negative luminal tumours (34-37) and that menopausal hormone use may increase the risk for hormone receptor positive tumours (38-40).

The Norwegian Breast Cancer Screening Program was implemented in Nord-Trøndelag County in 2001. It entails biennial screening of women aged 50-69 years. Women in this study who were born before 1929 were not eligible for the screening program, and some of the higher incidence of HER2 negative luminal tumours that we found in women born after 1929 could be due to a combination of increased unsystematic use of mammography for screening purposes during the 1990s (41, 42), and later implementation of organized mammography screening.

Between 1987 and 2001, use of menopausal hormone therapy increased greatly in Norway, after which an increase in hormone receptor positive tumours (ER and/or PR >10%) was observed (41). The use of hormone therapy declined after 2001 (43, 44).

The observed increase in use of menopausal hormone therapy concurred with increased use of mammography for screening purposes. Therefore, some of the higher incidence of Luminal A and Luminal B (HER2-) tumours observed for women born between 1929 and 1977 may be attributed to mammography screening and menopausal hormone therapy (3, 41, 45), both of which were negligible exposures in women born before 1929.

The impact of risk factors seems to differ between molecular subtypes, and it is possible that the higher incidence of HER2 negative luminal tumours among women born in 1929 or later may also be explained by differences in reproductive and lifestyle factors, such as age at menarche, age at first birth, parity, age at menopause, and body mass index (39, 40, 46, 47).

Some tumours were unavailable for subtyping (34% of cases born before 1929, and 9% of cases born in 1929 or later), mainly because patients were diagnosed at other hospitals. We therefore used multiple imputations to compensate for the resulting underestimation of subtype-specific incidence rates. Even when all clinical information available is included in the imputation models, it is difficult to assess how well the imputed rates reflect the true rates for each subtype. This uncertainty is also reflected in the relatively wide confidence intervals for the imputed rates. Although weaker after imputation, the differences in incidence rates persisted for the HER2 negative luminal subtypes, whereas the observed differences for Luminal B (HER+) and non-luminal subtypes disappeared after imputation for unknown subtype. Imputations had stronger effects on the subtype-specific rates for women born before 1929, due to a higher frequency of unknown subtype among these women.

Breast cancer mortality in Norway has declined since the mid-1990s, and this has been attributed to earlier detection (48, 49), and improved treatment (50-52). We found that the prognosis was generally better for women diagnosed with breast cancer in 1995 or later, compared to before 1995, confirming the findings of others (6, 23, 53).

Differences in prognosis (9, 54) and treatment response (55, 56) between subtypes have been demonstrated, and in accordance with others, we found clear reductions in case fatality for HER2 negative luminal subtypes from the first to the second diagnostic period (6, 53). We also found clear reductions in case fatality for the Basal phenotype.

The HER2 type had the worst prognosis irrespective of diagnostic period, and compared to Luminal A, the relative rate of death from HER2 type increased dramatically from the first to the second diagnostic period. This increase could probably be attributed to longer survival among Luminal A patients diagnosed in 1995 or later. Since Luminal tumours are more likely to be detected by screening (34-36), it is plausible that the longer survival among many Luminal A cases diagnosed after 1995 may be due to earlier detection by

mammography (lead-time bias). Aggressive subtypes, such as the Basal phenotype or the HER2 type, are more likely to present clinically, and lead-time bias may be a negligible issue for these subtypes (34-37).

Contrary to others (23), we could not demonstrate clear improvements in survival for the HER2 type between diagnostic periods. One possible explanation could be that targeted treatment with trastuzumab was not implemented until the last years of the observation period.

In conclusion, there has been a dramatic secular increase in the incidence rates of Luminal A and Luminal B (HER2-) breast cancer, whereas the incidence of Luminal B (HER2+) and non-luminal subtypes have remained relatively stable. The prognoses for Luminal A, Luminal B (HER2-), and Basal phenotype have clearly improved after 1995.

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Table 1. Incidence rates and incidence rate ratios of breast cancer molecular subtypes according to age at diagnosis and year of birth. Observed and imputed estimates.

		Observed				Imputed[a]			
		Incidence rate (cases/100 000 person-years)				Incidence rate (cases/100 000 person-years)			
Molecular subtype	**Age**	**Women born 1886-1928**	**Women born 1929-1977**	**IRR**	**(95% CI)**	**Women born 1886-1928**	**Women born 1929-1977**	**IRR**	**(95% CI)**
Total[b]	50-54	97.3	195.7	2.1	(1.5-2.8)				
	55-59	122.6	213.2	1.7	(1.3-2.3)				
	60-64	149.5	309.4	2.1	(1.6-2.7)				
	65-69	179.7	235.5	1.3	(1.0-1.7)				
Luminal A	50-54	9.9	76.1	7.7	(3.4-17.4)	24.3	84.7	3.5	(1.8-6.9)
	55-59	17.7	118.4	6.7	(3.8-11.9)	34.9	132.3	3.8	(2.3-6.4)
	60-64	35.5	158.9	4.5	(2.9-6.8)	54.9	177.3	3.2	(2.2-4.8)
	65-69	60.9	142.0	2.3	(1.6-3.5)	86.4	154.3	1.8	(1.2-2.6)
Luminal B (HER2-)	50-54	8.5	50.0	5.9	(2.4-14.5)	23.1	57.6	2.5	(1.2-5.2)
	55-59	25.9	37.9	1.5	(0.8-2.8)	45.3	44.7	1.0	(0.5-1.9)
	60-64	19.9	66.9	3.4	(1.8-6.2)	37.5	71.9	1.9	(1.1-3.4)
	65-69	32.4	38.1	1.2	(0.6-2.3)	49.5	41.6	0.8	(0.4-1.6)
Luminal B (HER2+)	50-59	9.6	10.2	1.1	(0.5-2.4)	17.6	13.8	0.8	(0.4-1.7)
	60-69	8.1	21.6	2.7	(1.3-5.5)	14.0	23.1	1.7	(0.8-3.5)
HER2 type	50-59	8.3	11.3	1.4	(0.6-3.1)	18.2	13.9	0.8	(0.4-1.6)
	60-69	9.1	7.7	0.8	(0.3-2.3)	15.1	9.4	0.6	(0.2-1.6)
5 negative phenotype	50-59[c]	-	-	-	-	-	-	-	-
	60-69	5.6	7.7	1.4	(0.5-4.0)	10.1	9.1	0.9	(0.3-2.6)
Basal phenotype	50-59	4.5	13.6	3.0	(1.2-7.8)	8.9	16.2	1.8	(0.8-4.3)
	60-69	7.1	7.7	1.1	(0.4-3.0)	11.1	9.0	0.8	(0.3-2.6)

Abbreviations: IRR=Incidence rate ratio, CI=Confidence interval, HER2=Human epidermal growth factor receptor 2

[a] Based on 50 imputed datasets using age (5-year categories) and calendar year at diagnosis (continuous), stage (I, II, III, IV, unknown) and extent of disease (disease localized to the breast, local invasion, regional lymph nodes, distant lymph nodes or organ metastases, unknown) as reported by the Cancer Registry of Norway, year of birth (5-year categories), observation time after diagnosis (log-transformed, continuous) and survival status (alive, death from breast cancer, death from other causes). [b] Total breast cancer incidence from the Cancer Registry of Norway, including cases with unknown subtype. [c] Too few observations.

Table 2: Characteristics of breast cancer cases with successfully subtyped tumours.		
Women with incident breast cancer	**Diagnosis before 1995**	**Diagnosis in 1995 or later**
Number of women	661	762
Mean age at diagnosis (SD)	69.5 (10.4)	65.5 (14.3)
Mean follow-up after diagnosis (SD)	9.8 (8.7)	7.9 (4.4)
Deaths from breast cancer (%)	293 (44)	131 (17)
Deaths from other causes (%)	316 (48)	145 (19)
Molecular subtype (%)		
Luminal A	291 (44)	414 (54)
Luminal B (HER2-)	194 (29)	183 (24)
Luminal B (HER2+)	55 (8)	57 (7)
HER2 type	53 (8)	36 (5)
5 negative phenotype	23 (3)	25 (3)
Basal phenotype	45 (7)	47 (6)
Histopathological grade (%)		
1	78 (12)	145 (19)
2	346 (52)	397 (52)
3	237 (36)	220 (29)
Unknown	-	-
Regional lymph node metastasis (%)		
Yes	234 (35)	239 (31)
No	238 (35)	418 (55)
Unknown histopathology[a]	189 (29)	105 (14)
Tumor size (%)		
≤2 cm	268 (41)	466 (61)
>2 cm-5 cm	27 (4)	236 (31)
>5 cm	9 (1)	29 (4)
Uncertain, but >2 cm	141 (21)	4 (1)
Uncertain	216 (33)	27 (4)
Stage (%)[b]		
I	338 (51)	390 (51)
II	239 (36)	314 (41)
III	43 (7)	35 (5)
IV	35 (5)	23 (3)
Unknown	6 (1)	-
Extent of disease (%)[b]		
Disease localized to the breast	225 (34)	369 (48)
Local invasion	23 (3)	14 (2)
Regional lymph nodes	155 (23)	234 (31)
Distant lymph node or organ metastases	25 (4)	22 (3)
Unknown	233 (35)	123 (16)
Abbreviations: SD=Standard deviation, HER2=Human epidermal growth factor receptor 2		

[a] Includes cases where histopathological examination was done, but reports were not available, and cases where no axillary lymph nodes were removed.

[b] As recorded by the Cancer Registry of Norway. Information is based on histopathological and/or clinical examination.

Table 3. Absolute and relative risk of death from breast cancer according to molecular subtype and diagnostic period.

Molecular subtype	Patients (n)	Cumulative incidence of death from breast cancer				Age-adjusted hazard ratio of death from breast cancer [a]		
		First 5 years after diagnosis		First 15 years after diagnosis		Total follow-up time after diagnosis		
		Deaths (n)	Cum. inc. %, (95% CI)	Deaths (n)	Cum. inc. %, (95% CI)	Deaths (n)	Within period HR (95% CI)	Between periods HR (95% CI)[b]
Women diagnosed before 1995								
Luminal A	291	54	19 (15-24)	103	37 (32-44)	112	1.0	1
Luminal B (HER2-)	194	45	23 (18-30)	79	42 (35-49)	87	1.3 (1.0-1.7)	1
Luminal B (HER2+)	55	20	36 (25-51)	25	46 (34-60)	28	1.3 (0.9-2.0)	1
HER2 type	53	27	51 (38-65)	30	57 (44-71)	31	2.3 (1.5-3.5)	1
5 negative phenotype	23	10	43 (26-66)	13	57 (38-77)	13	1.7 (1.0-3.1)	1
Basal phenotype	45	18	40 (27-56)	22	50 (36-65)	22	1.6 (1.0-2.5)	1
Women diagnosed in 1995 or later								
Luminal A	414	30	7 (5-10)	44	13 (9-17)	44	1.0	0.4 (0.3-0.5)
Luminal B (HER2-)	183	25	14 (10-20)	34	23 (16-32)	34	2.0 (1.2-3.1)	0.5 (0.3-0.7)
Luminal B (HER2+)	57	11	20 (11-32)	18	42 (27-63)	18	3.6 (2.1-6.3)	0.7 (0.4-1.3)
HER2 type	36	13	36 (23-54)	15	42 (28-60)	15	5.1 (2.8-9.3)	0.6 (0.3-1.1)
5 negative phenotype	25	8	32 (17-54)	9	36 (21-58)	9	4.2 (2.0-8.6)	0.6 (0.3-1.6)
Basal phenotype	47	9	20 (11-34)	11	26 (15-42)	11	2.7 (1.4-5.2)	0.4 (0.2-0.9)

Abbreviations: Cum. inc.=Cumulative incidence, HR=Hazard ratio, CI=Confidence interval, HER2=Human epidermal growth factor receptor 2

[a]HR from Cox regression, adjusted for age (45-49, 50-59, 60-64, 65-69, 70-74, 75+ years). Adjustments for grade or stage of disease did not substantially influence the results.
[b]Diagnosis before 1995 was used as the reference.

Figure 1

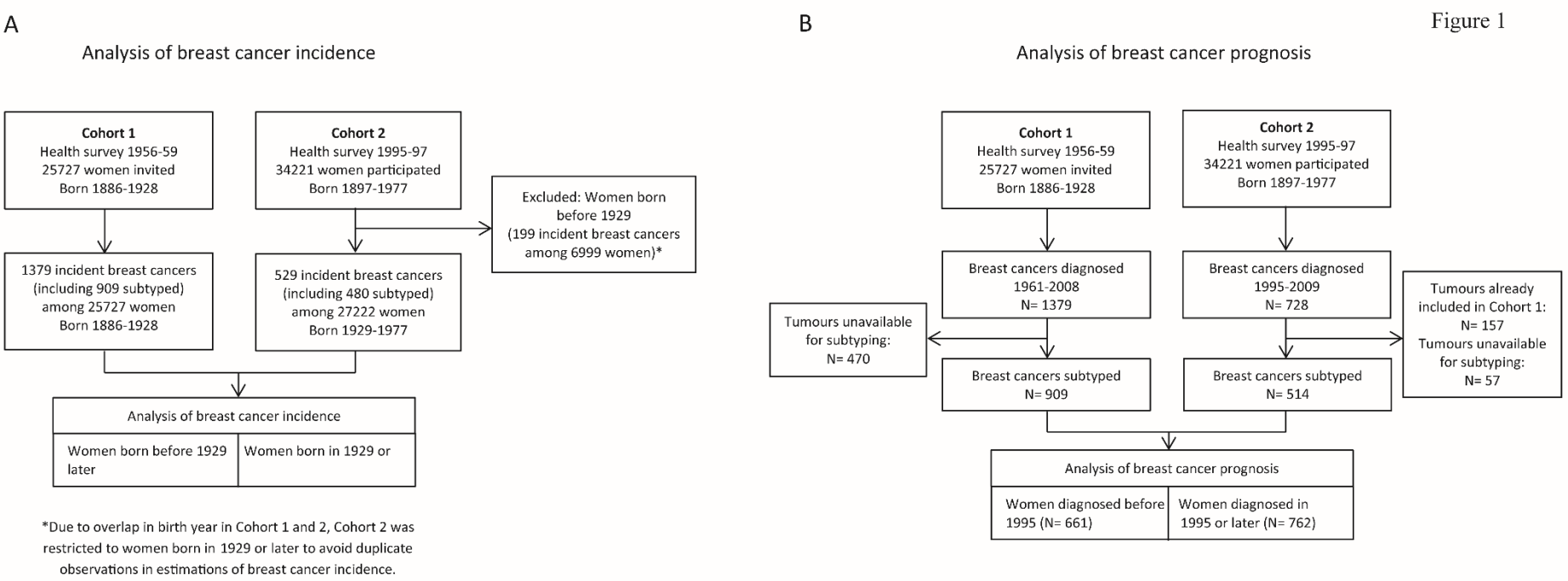

A
Analysis of breast cancer incidence
Cohort 1
Health survey 1956-59
25727 women invited
Born 1886-1928
Cohort 2
Health survey 1995-97
34221 women participated
Born 1897-1977
Excluded: Women born before 1929
(199 incident breast cancers among 6999 women)*
1379 incident breast cancers (including 909 subtyped) among 25727 women Born 1886-1928
529 incident breast cancers (including 480 subtyped) among 27222 women Born 1929-1977
Analysis of breast cancer incidence
Women born before 1929 later
Women born in 1929 or
*Due to overlap in birth year in Cohort 1 and 2, Cohort 2 was restricted to women born in 1929 or later to avoid duplicate observations in estimations of breast cancer incidence.
B
Analysis of breast cancer prognosis
Cohort 1
Health survey 1956-59
25727 women invited
Born 1886-1928
Cohort 2
Health survey 1995-97
34221 women participated
Born 1897-1977
Breast cancers diagnosed 1961-2008 N= 1379
Breast cancers diagnosed 1995-2009 N= 728
Tumours unavailable for subtyping: N= 470
Tumours already included in Cohort 1: N= 157
Tumours unavailable for subtyping: N= 57
Breast cancers subtyped N= 909
Breast cancers subtyped N= 514
Analysis of breast cancer prognosis
Women diagnosed before 1995 (N= 661)
Women diagnosed in 1995 or later (N= 762)

Figure 2

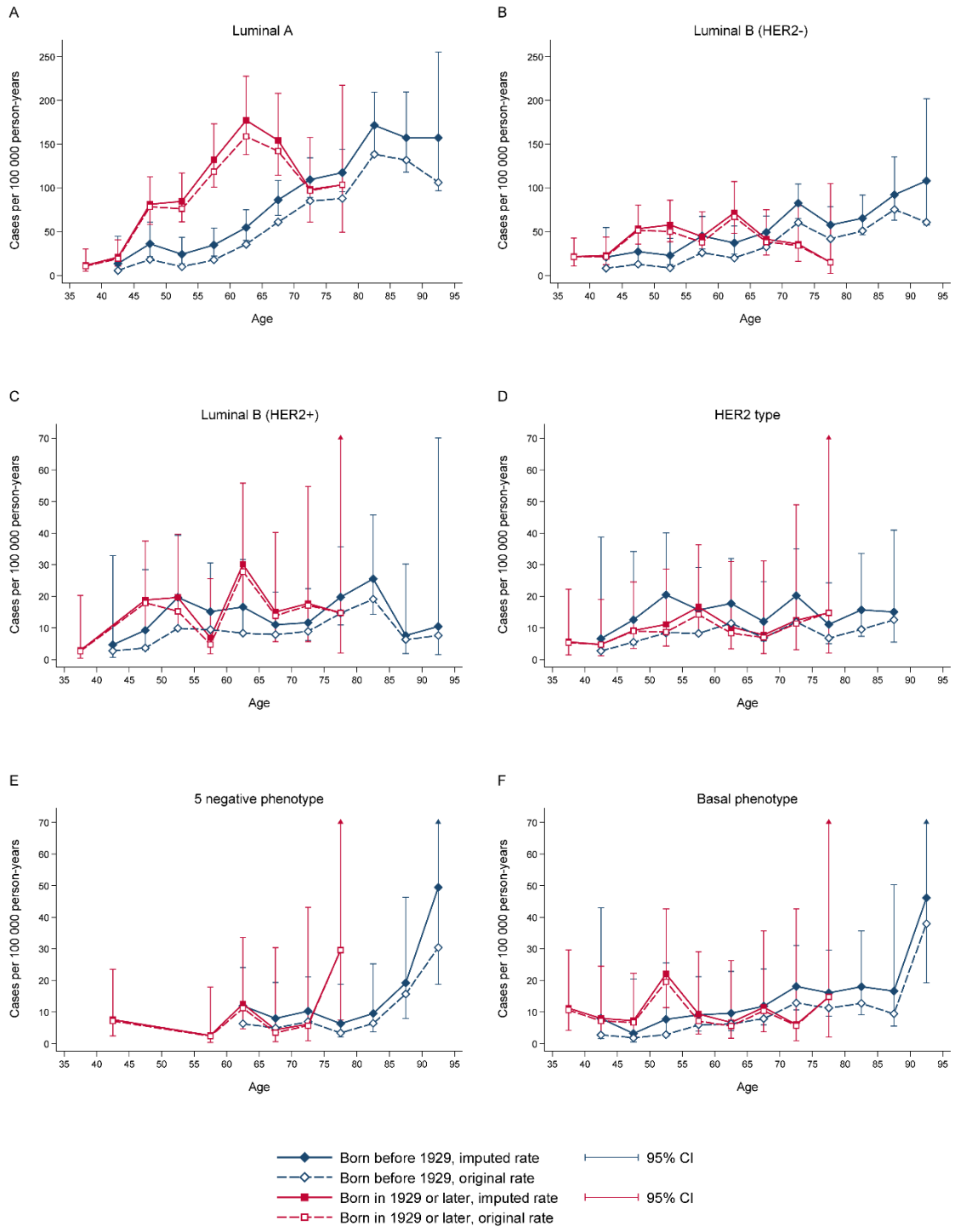
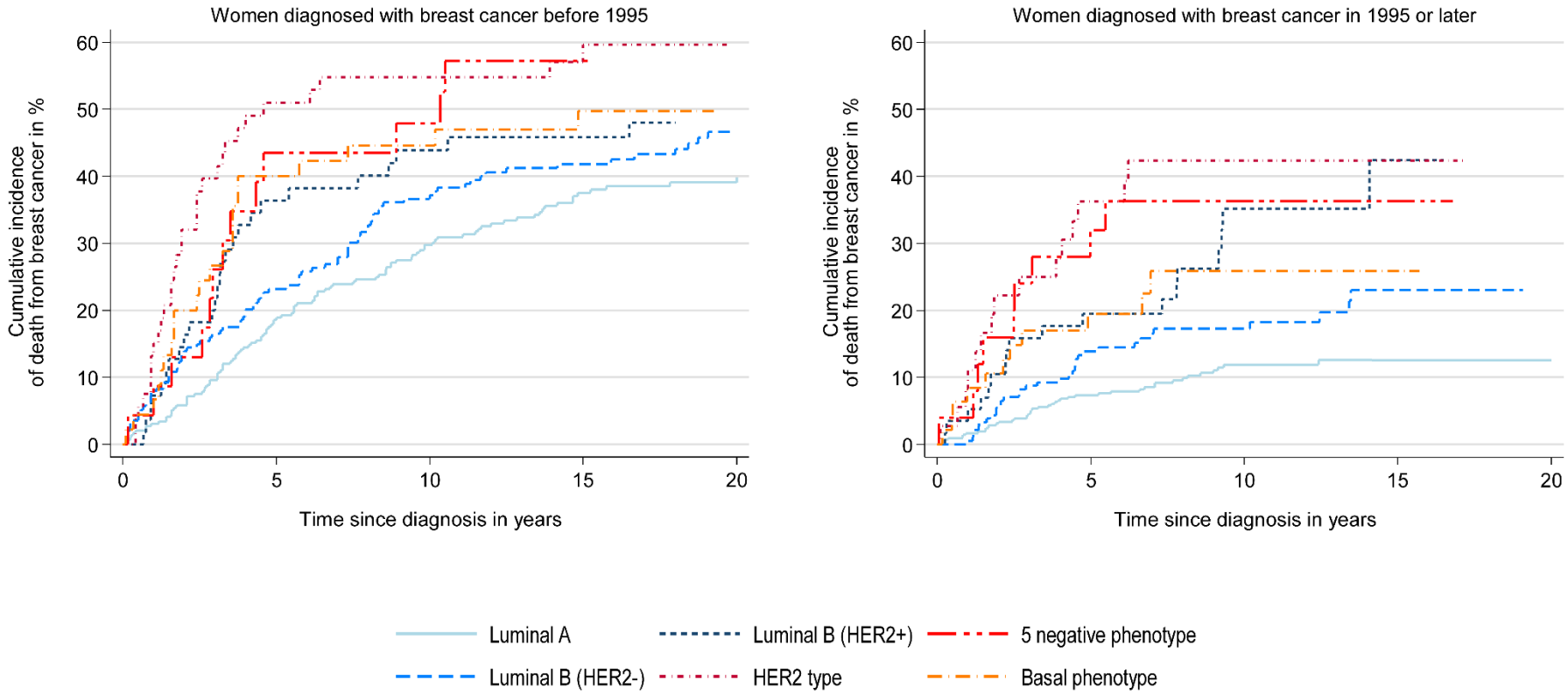
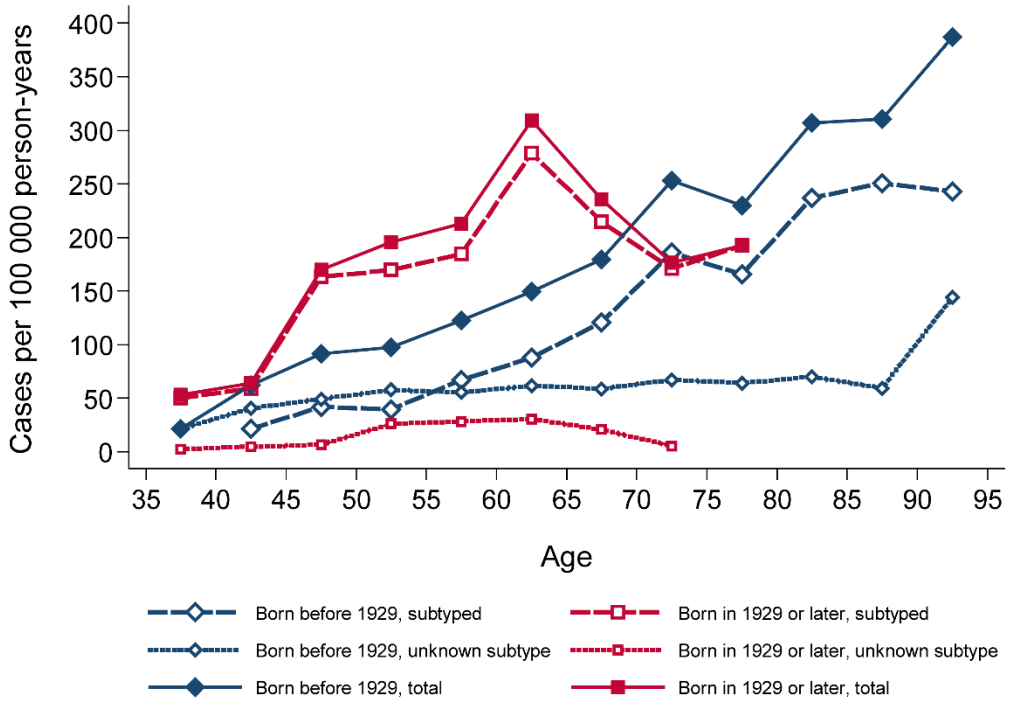

A
Luminal A
B
Luminal B (HER2-)
C
Luminal B (HER2+)
D
HER2 type
E
5 negative phenotype
F
Basal phenotype
Cases per 100 000 person-years
Age
Born before 1929, imputed rate
Born before 1929, original rate
Born in 1929 or later, imputed rate
Born in 1929 or later, original rate
95% CI
95% CI

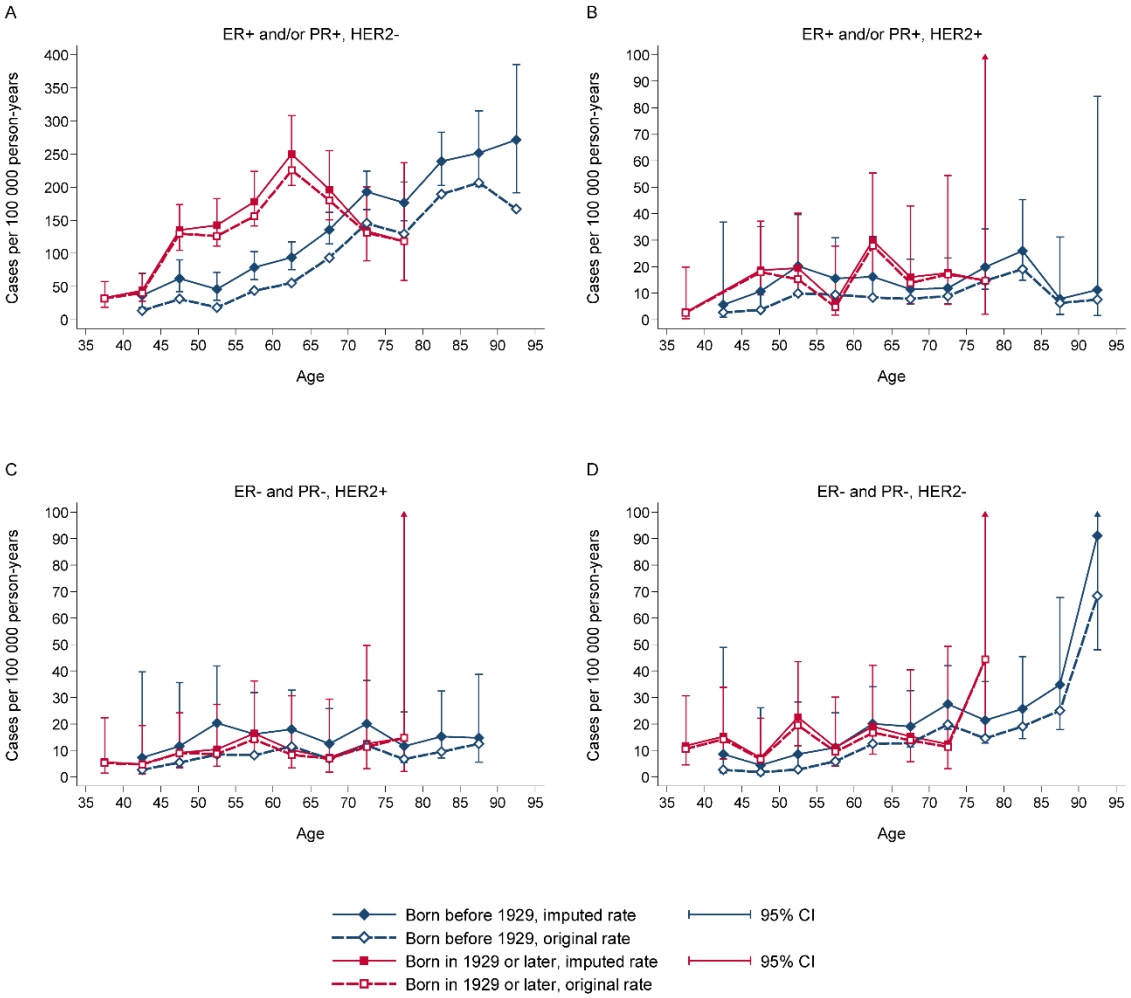
Figure 3


Women diagnosed with breast cancer before 1995
Women diagnosed with breast cancer in 1995 or later
Cumulative incidence of death from breast cancer in %
0
10
20
30
40
50
60
0
5
10
15
20
Time since diagnosis in years
Luminal A
Luminal B (HER2-)
Luminal B (HER2+)
HER2 type
5 negative phenotype
Basal phenotype

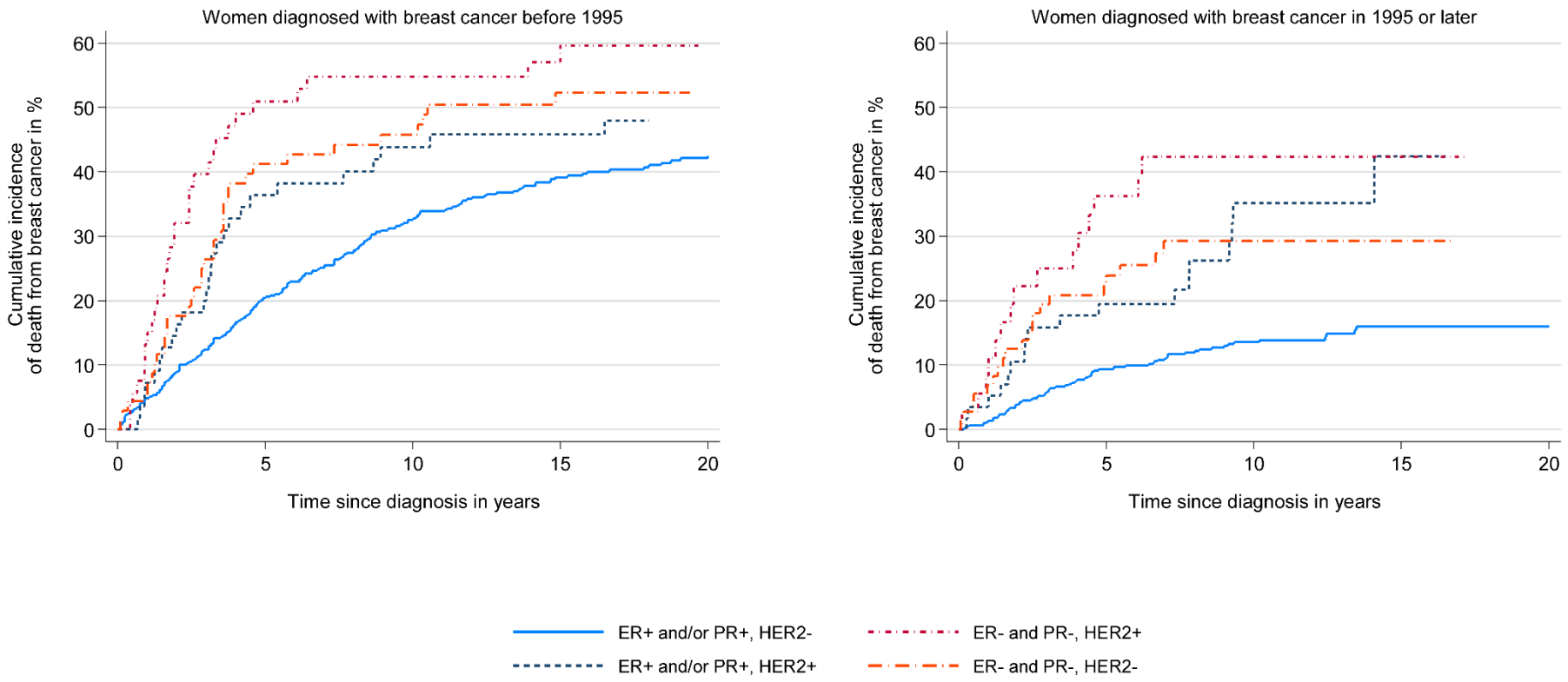
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Titles and legends to figures

Figure 1. Overview of study population. A: Analysis of breast cancer incidence. B: Analysis of breast cancer prognosis.

Figure 2. Subtype-specific breast cancer incidence rates according to age and year of birth. Blue lines: Women born before 1929. Red lines: Women born in 1929 or later. Dotted lines (red and blue) represent incidence rates of subtyped cases. Solid lines (red and blue) represent average incidence rates from 50 imputed datasets with corresponding 95% confidence intervals (CIs). A) Luminal A, B) Luminal B (HER2-), C) Luminal B (HER2+), D) HER2 type, E) 5 negative phenotype, and F) Basal phenotype.

Figure 3. Cumulative incidence of death from breast cancer according to molecular subtypes. A: Women diagnosed before 1995 (Gray's test: p=0.0004). B: Women diagnosed in 1995 or later (Gray's test: p=<0.0001).

Titles and legends to Supplementary Figures.

Supplementary Figure 1. Breast cancer incidence rates according to age and year of birth. Blue lines: Women born before 1929. Red lines: Women born in 1929 or later. Dashed lines (red and blue) represent incidence rates of subtyped cases. Dotted lines (red and blue) represent incidence rates of cases with unknown subtype. Solid lines (red and blue) represent the total incidence rates (subtyped cases and cases with unknown subtype combined).

Supplementary Figure 2. Subtype-specific incidence rates according to age and year of birth. Blue lines: Women born before 1929. Red lines: Women born in 1929 or later. Dotted lines (red and blue) represent incidence rates of subtyped cases. Solid lines (red and blue) represent average incidence rates from 50 imputed datasets with corresponding 95% confidence intervals (CIs).

Supplementary Figure 3. Cumulative incidence of death from breast cancer according to breast cancer subtypes. A: Women diagnosed before 1995 (Gray's test: p=0.0002). B: Women diagnosed in 1995 or later (Gray's test: p<0.0001).

Supplementary Table 1: Characteristics of the study population used in estimations of breast cancer incidence

	Cohort 1		Cohort 2[a]	
Women followed for breast cancer occurrence	**Women born 1886-1928**		**Women born 1929-1977**	
Number of women	25 727		27 222	
Mean age at baseline (SD)	51.0 (11.6)		43.4 (12.8)	
Mean duration of follow-up (SD)	29.7 (13.9)		13.1 (1.7)	
Number of incident breast cancers	1379		529	
Women with incident breast cancer	**Subtyped**	**Not subtyped**	**Subtyped**	**Not subtyped**
Number of cases	909	470	480	49
Mean age at diagnosis (SD)	73.0 (10.7)	67.8 (12.9)	57.3 (9.5)	56.7 (7.9)
Mean follow-up after diagnosis (SD)	8.8 (8.0)	9.5 (10.5)	8.9 (4.0)	8.1 (4.4)
Deaths from breast cancer (%)	359 (39)	242 (51)	54 (11)	9 (18)
Deaths from other causes (%)	413 (45)	199 (42)	38 (8)	3 (6)
Molecular subtype (%)				
Luminal A	433 (48)	-	255 (53)	-
Luminal B (HER2-)	248 (27)	-	121 (25)	-
Luminal B (HER2+)	71 (8)	-	37 (8)	-
HER2 type	62 (7)	-	26 (5)	-
5 negative phenotype	33 (4)	-	12 (3)	-
Basal phenotype	62 (7)	-	29 (6)	-
Stage (%)[b]				
I	455 (50)	216 (46)	260 (54)	32 (65)
II	346 (38)	137 (29)	194 (40)	11 (22)
III	57 (6)	36 (8)	15 (3)	1 (2)
IV	45 (5)	71 (15)	11 (2)	5 (10)
Unknown	6 (1)	10 (2)	-	-
Extent of disease (%)[b]				
Disease localized to the breast	309 (34)	192 (41)	267 (56)	29 (59)
Local invasion	30 (3)	12 (3)	3 (1)	-
Regional lymph nodes	229 (25)	134 (29)	155 (32)	11 (22)
Distant lymph node or organ metastases	35 (4)	64 (14)	10 (2)	4 (8)
Unknown	306 (34)	68 (14)	45 (9)	5 (10)

Abbreviations: SD=Standard deviation, HER2=Human epidermal growth factor receptor 2

[a]In estimations of breast cancer incidence, Cohort 2 was restricted to women born after 1928 in order to avoid duplicate observations.

[b] As recorded by the Cancer Registry of Norway. Information is based on histopathological and/or clinical examination.

Supplementary Table 2. Incidence rates and incidence rate ratios of breast cancer according to hormone and HER2 receptor status, age at diagnosis and year of birth. Observed and imputed estimates.

		Observed				Imputed[a]			
		Incidence rate (cases/100 000 person-years)				Incidence rate (cases/100 000 person-years)			
Breast cancer subtype	**Age**	**Women born 1886-1928**	**Women born 1929-1977**	**IRR**	**95% CI**	**Women born 1886-1928**	**Women born 1929-1977**	**IRR**	**95% CI**
Total[b]	50-54	97.3	195.7	2.1	(1.5-2.8)				
	55-59	122.6	213.2	1.7	(1.3-2.3)				
	60-64	149.5	309.4	2.1	(1.6-2.7)				
	65-69	179.7	235.5	1.3	(1.0-1.7)				
ER+ and/or PR+, HER2-	50-54	18.3	126.1	6.9	(3.8-12.6)	45.9	142.6	3.1	(1.9-5.1)
	55-59	43.6	156.3	3.6	(2.4-5.4)	78.8	177.9	2.3	(1.6-3.2)
	60-64	55.4	225.8	4.1	(2.9-5.8)	94.2	249.8	2.7	(2.0-3.6)
	65-69	93.3	180.1	1.9	(1.4-2.7)	135.9	196.4	1.4	(1.1-2.0)
ER+ and/or PR+, HER2+	50-59	9.6	10.2	1.1	(0.5-2.4)	17.9	13.6	0.8	(0.3-1.7)
	60-69	8.1	21.6	2.7	(1.3-5.5)	13.9	23.8	1.7	(0.8-3.5)
ER- and PR-, HER2+	50-59	8.3	11.3	1.4	(0.6-3.1)	18.3	13.4	0.7	(0.3-1.6)
	60-69	9.1	7.7	0.8	(0.3-2.3)	15.3	9.0	0.6	(0.2-1.5)
ER- and PR-, HER2-	50-59	4.5	14.7	3.3	(1.3-8.2)	10.2	17.2	1.7	(0.7-4.0)
	60-69	12.7	15.4	1.2	(0.6-2.5)	19.7	17.4	0.9	(0.4-1.8)

Abbreviations: IRR=Incidence rate ratio, CI=Confidence interval, HER2=Human epidermal growth factor receptor 2

[a] Based on 50 imputed datasets using age (5-year categories) and calendar year at diagnosis (continuous), stage (I, II, III, IV, unknown) and extent of extent of disease (disease localized to the breast, local invasion, regional lymph nodes, distant lymph nodes or organ metastases, unknown) as reported by the Cancer Registry of Norway, year of birth (5-year categories), observation time after diagnosis (log-transformed, continuous) and survival status (alive at end of follow-up, death from breast cancer, death from other causes).

[b] Total breast cancer incidence from the Cancer Registry of Norway, including cases with unknown subtype.

Supplementary Table 3. Risk of death from breast cancer between diagnostic periods, for each molecular subtype 5 and 15 years after diagnosis (Cox regression analysis).				
Molecular subtype	**1995 or later vs. before 1995[a]**			
	First 5 years after diagnosis		**First 15 years after diagnosis**	
	HR[b]	**95% CI**	**HR[b]**	**95% CI**
Luminal A	0.4	0.3-0.7	0.4	0.3-0.5
Luminal B (HER2-)	0.6	0.3-0.9	0.5	0.3-0.7
Luminal B (HER2+)	0.5	0.2-1.0	0.7	0.4-1.4
HER2 type	0.6	0.3-1.1	0.6	0.3-1.1
5 negative phenotype	0.9	0.3-2.4	0.6	0.3-1.6
Basal phenotype	0.4	0.2-0.8	0.4	0.2-0.9
Abbreviations: HR=Hazard ratio, CI=Confidence interval, HER2=Human epidermal growth factor receptor 2				

[a]Diagnosis before 1995 was used as the reference.

[b]HR from Cox regression, adjusted for age (≤49, 50-59, 60-64, 65-69, 70-74, 75+ years). Adjustments for grade or stage of disease did not substantially influence the results.

Supplementary Table 4. Absolute and relative risk of death from breast cancer according to breast cancer subtype and diagnostic period.

Molecular subtype		Cumulative incidence of death from breast cancer				Age-adjusted hazard ratio of death from breast cancer [a]		
		First 5 years after diagnosis		First 15 years after diagnosis		Total follow-up time after diagnosis		
	Patients (n)	Deaths (n)	Cum. inc. %, (95% CI)	Deaths (n)	Cum. inc. %, (95% CI)	Deaths (n)	Within period HR (95% CI)	Between periods HR (95% CI)[b]
Women diagnosed before 1995								
ER+ and/or PR+, HER2-	485	99	20 (17-24)	182	39 (35-44)	199	1.0	1
ER+ and/or PR+, HER2+	55	20	36 (25-51)	25	46 (34-60)	28	1.2 (0.8-1.8)	1
ER- and PR-, HER2+	53	27	51 (38-65)	30	57 (44-71)	31	2.1 (1.4-3.0)	1
ER- and PR-, HER2-	68	28	41 (31-54)	35	52 (41-65)	35	1.4 (1.0-2.1)	1
Women diagnosed in 1995 or later								
ER+ and/or PR+, HER2-	597	55	9 (7-12)	78	16 (13-20)	78	1.0	0.4 (0.3-0.5)
ER+ and/or PR+, HER2+	57	11	20 (11-32)	18	42 (27-63)	18	2.8 (1.7-4.7)	0.7 (0.4-1.3)
ER- and PR-, HER2+	36	13	36 (23-54)	15	42 (28-60)	15	4.0 (2.3-7.0)	0.6 (0.3-1.1)
ER- and PR-, HER2-	72	17	24 (16-37)	20	29 (20-42)	20	2.5 (1.5-4.1)	0.5 (0.3-0.9)

Abbreviations: Cum. inc.= Cumulative incidence, HR=Hazard ratio, CI=Confidence interval, HER2=Human epidermal growth factor receptor 2

[a]HR from Cox regression, adjusted for age (≤49, 50-59, 60-64, 65-69, 70-74, 75+ years). Adjustments for grade or stage of disease did not substantially influence the results.

[b]Diagnosis before 1995 was used as the reference.

Supplementary Table 5. Risk of death from breast cancer between diagnostic periods, for each breast cancer subtype 5 and 15 years after diagnosis (Cox regression analysis).

Breast cancer subtype	1995 or later vs. before 1995[a]			
	First 5 years after diagnosis		First 15 years after diagnosis	
	HR[b]	95% CI	HR[b]	95% CI
ER+ and/or PR+, HER2-	0.4	0.3-0.6	0.4	0.3-0.5
ER+ and/or PR+, HER2+	0.5	0.2-1.0	0.7	0.4-1.4
ER- and PR-, HER2+	0.6	0.3-1.1	0.6	0.3-1.1
ER- and PR-, HER2-	0.5	0.3-1.0	0.5	0.3-0.9

Abbreviations: HR=Hazard ratio, CI=Confidence interval, HER2=Human epidermal growth factor receptor 2

[a]Diagnosis before 1995 was used as the reference.

[b]HR from Cox regression, adjusted for age (≤49, 50-59, 60-64, 65-69, 70-74, 75+ years). Adjustments for grade or stage of disease did not substantially influence the results.