Article Type : Original Article

Invasive lobular breast cancer: The prognostic impact of histopathological grade, E-cadherin and molecular subtypes.

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Abstract

Aims: The aim of the study was to compare breast cancer specific survival (BCSS) for invasive lobular carcinoma (ILC) and invasive ductal carcinoma (IDC). Further, to critically evaluate the prognostic value of histopathological grading of ILC and to examine E-cadherin as a prognostic marker in ILC.

Methods: The study comprised 116 lobular and 611 ductal breast carcinomas occurring between 1961 and 2008. All cases had previously been classified according to histopathological type and grade, stained for ER, PR, Ki67, EGFR, CK5 and HER2 and classified into molecular subtypes. For the present study, immunohistochemical staining for E-cadherin was done. Kaplan-Meier method and Cox proportional hazards models were used in the analyses.

Results: Grade 2 tumours comprised 85.3 % of the lobular tumours and 51.9 % of the ductal tumours. BCSS in ILC grade 2 was comparable to that of IDC grade 3. E-cadherin negative

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as an 'Accepted Article', doi: 10.1111/his.12572

ILC had a poorer prognosis compared to E-cadherin positive ILC and to IDC regardless of E-cadherin status.

Conclusions: The implication of histopathological grading may differ in ILC compared to IDC. E-cadherin may be useful in prognostication in ILC and thereby influence the determination of treatment strategies for this group of women.

Introduction

Invasive lobular carcinoma (ILC) is defined as an invasive carcinoma comprising non-cohesive cells individually dispersed in a single-file linear pattern in a fibrous stroma and accounts for 5-15 % of breast cancers[1-3]. There are a number of variants of ILC that do not show the classical morphological pattern but loss of cell-to-cell cohesion is a common feature [3].

Histopathological grade is an important prognostic tool[4-6]. The Nottingham grading system classifies patients into groups with different prognoses[7]. However, in ILC the suitability of grading is uncertain[8, 9]. Glandular structures are absent, mitoses are infrequent and the nuclei uniform. Thus, most ILCs are grade 2 and the prognostic value of grading is unclear.

Breast cancer treatment guidelines are based on hormone receptor-, human epidermal growth factor receptor 2 (HER2)-, and proliferation (Ki67) status, in addition to histopathological grade, tumour size and lymph node status [10]. Histopathological type is not always included as a parameter in treatment guidelines, though favourable types may influence the choice of treatment.

E-cadherin (E-cad) is a transmembrane protein involved in cell-to-cell adhesion, and its loss promotes invasion and metastasis[11]. Loss of E-cad is common in ILC [11, 12], and supports the

diagnosis of ILC [13]. Although it has been suggested that low levels of E-cad are associated with poorer prognosis[14-16], its potential as a prognostic marker in ILC has not been clarified.

The aims of this study were to compare breast cancer specific survival (BCSS) in ILC with invasive ductal carcinoma (IDC) in a cohort of breast cancer patients with long follow-up, to assess the prognostic value of histopathological grading of ILC and to examine the potential of E-cad as a prognostic marker in ILC.

Material and methods

Study population

Between 1956 and 1959, women from Nord Trøndelag County in Norway were invited by the Norwegian Cancer Registry to participate in a breast cancer survey. The population has been described previously[17, 18]. Briefly, 25 897 women, born between 1886 and 1928 were invited. From1961 to 2008, 1393 developed breast cancer. Cases occurring prior to 1961 were excluded. A total of 945 tissue samples were available at the Department of Pathology and Medical Genetics, St. Olav's Hospital, Trondheim, Norway, and 867 were suitable for inclusion in tissue microarrays (TMA). After linkage with the Cause of Death Registry of Norway and the Norwegian Cancer Registry, survival data were generated. Only cases of IDC of no special type and ILC (727 cases) were included in the present study.

Specimen Characteristics

All cases were classified into histopathological type and grade and reclassified into molecular subtypes using surrogate markers for gene expression analyses (Figure 1) [17].
Histopathological typing and grading was done on full-face sections independently by two

experienced pathologists (OAH, AMB) [3 5, 19]. Three 1mm tissue cores from the periphery of each tumour were selected and assembled in TMAs. Immunohistochemical (IHC) staining was done for oestrogen receptor (ER), progesterone receptor (PR), Ki67, HER2, cytokeratin 5 (CK5) and epithelial growth factor receptor 1 (EGFR). *HER2* gene amplification status was estimated using chromogenic in situ hybridization (CISH). For the present study, IHC staining for E-cad was done.

Assay methods

Assay methods for all markers except E-cad are described in detail previously [17]. For the present study, IHC for detection of E-cad was performed according to the manufacturer`s guidelines (Dako). The sections were mounted on Superfrost+ glass slides, dried at 37° C overnight and stored at -20°C. Before staining, the slides were heated to 60°C for 2hrs and pre-treated in a PT Link, Pre-Treatment Module for Tissue Specimens (Dako) with buffer (High pH Target Retrieval Solution K8004) at 97°C for 20 min. Monoclonal mouse antibody (clone NCH-38), 55.2 mg/L dilution 1:100, was applied. For visualization, Dako REAL^{TM} EnVision^{TM} Detection System was used with Peroxidase/DAB+, Rabbit/Mouse, code K5007.

Scoring and reporting

The REMARK recommendations for tumour marker studies were followed [20]. All IHC evaluations were done by two researchers independently. ER and PR were positive if ≥1 % of the tumour cells showed positive nuclear staining. For Ki67, ≥15 % stained nuclei was classified as Ki67 high and <15 % as Ki67 low. A staining index (SI) (intensity x proportion) was calculated for CK5 and EGFR, and SI of 0–1 was considered to be negative and 2–9 was

considered to be positive as previously described. *HER2* gene amplification was defined as gene to chromosome ratio ≥ 2. In cases where CISH failed, +3 IHC staining for HER2 was recorded as positive [17]. In the present study, only moderate or strong continuous membrane staining for E-cad in > 50 % of tumour cells were classified as positive. There were very few cases with aberrant staining (cytoplasmic staining or intermittent membranous staining), and these were classified as negative.

Statistical analyses

Follow-up was from date of diagnosis until death or December 31, 2010. Kaplan-Meier methods were used to estimate BCSS for ILC grade 2 compared to IDC grades 1, 2 and 3, and for comparing survival of ILC and IDC grade 2, E-cad positive and -negative tumours. Grade 2 ILC and IDC were compared for each of the following biomarker categories separately: ER+, Ki67 low and HER2-. Comparison was made between ILC and IDC grade 2 tumours with the favourable biomarker profile (ER+ and HER2- and Ki67 low). BCSS for Luminal A and Luminal B (HER2-) subtypes were compared for ILC and IDC separately. Log rank test was used to compare survival curves, $p< 0.05$ was considered statistically significant. Cox proportional hazards models were used to estimate relative risks of death from breast cancer adjusted for age (5-year intervals), stage at diagnosis (I, II, III, IV, unknown) and time period of diagnosis. Hazard ratios (HR) for ILC compared to IDC were calculated with 95 % confidence intervals (CI). The numbers of cases of ILC grades 1 and 3 were too low for reliable analyses of grade and BCSS in ILC. The number of cases with an unfavourable biomarker profile (ER-, HER2+ and Ki67 high) was too small for separate analysis (n=39). Statistical analyses were done using Stata version 12.1 (Stata Corp.).

Ethics

Approval was granted by the Regional Committee for Medical and Health Sciences Research Ethics including dispensation from the requirement of patient consent (REK, Midt-Norge, ref. nr: 836/2009).

Results

Description of the population

Of the 727 cases, 16 % were ILC and 84 % were IDC (Table 1). During follow-up 297 (40.9 %) died from breast cancer and 304 (41.8 %) died of other causes. At the end of the period, 126 (17.3 %) were still alive. Mean age at diagnosis was 71.3 years for IDC and 73.3 years for ILC. Table 2 shows the treatments given.

Tumour characteristics

Histopathological grade, tumour size, lymph node status, stage and molecular subtypes are given in Table 1. Table 3 shows the results of IHC and CISH. The proportion of histopathological grade 2 tumours was higher in ILC (85.3 %) compared to IDC (51.9 %). In ILC 87.9 % were ER+ and 6.0 % were HER2+, compared to 83.6 % ER+ and 16.9 % HER2+ in IDC. A higher proportion of ILC (16.4 %) than IDC (7.5 %) were >5 cm. However, the proportions of tumours between 2 and 5 cm were similar (42.2 % vs. 45.5 %).

Grade, type and prognosis

Figure 2 shows BCSS for ILC grade 2 compared to IDC grades 1, 2 and 3. ILC grade 2 had poorer BCSS compared to IDC grade 2 (p=0.01, Log-rank test).There was no significant difference in BCSS between ILC grade 2 and IDC grade 3 (p=0.48, Log-rank test). Table 4 shows the risk of death from breast cancer according to type. ILC grade 2 was compared to IDC grades 1, 2 and 3 separately. HRs were similar for ILC grade 2 and IDC grade 3, whereas IDC grade 2 had a significantly better survival than ILC grade 2 (HR 0.66, 95 % CI 0.46–0.94). Adjustment for age, stage and time of diagnosis did not influence the results.

Prognostic value of type in ER+, HER2- and Ki67 low tumours

Table 5 shows risk of death from breast cancer according to type among patients with grade 2 tumours and clinically favourable biomarker profiles. For each marker status (ER+, HER2-, Ki67 low) respectively there was a significantly higher risk of death from ILC compared to IDC. Similarly, risk of death from breast cancer for patients with grade 2 tumours expressing a complete favourable biomarker profile (ER+, HER2- and Ki67 low) was higher for ILC than IDC (HR 2.16, 95 % CI 1.34–3.49). Analysis of all grades did not alter the results (data not shown).

Prognostic value of molecular subtypes

The proportions of HER2+ and/or ER- ILC were low compared to IDC, as reflected in the distribution of molecular subtypes (Table 1). Among 353 Luminal A cases, 290 (82.2 %) were ductal and 63 (17.8 %) were lobular. Figure 3 shows that Luminal A ILC had a poorer prognosis than Luminal A IDC (p=0.02, Log-rank test). Luminal B (HER2-) IDC had a

slightly better prognosis than Luminal A and Luminal B (HER2-) ILC, (p=0.39, Log-rank test). Table 6 shows that risk of death from grade 2 breast cancer was higher for Luminal A ILC, Luminal B (HER2-) ILC and Luminal B (HER2-) IDC compared to Luminal A IDC. The difference between Luminal A IDC and ILC was statistically significant. The numbers in the other subtypes were too low for analysis.

Prognostic value of E-cadherin

Table 3 shows that 23.3 % of ILC were E-cad+. Figure 4 shows BCSS for grade 2 E-cad+ and E-cad- ILC and IDC. E-cad- ILC had poorer prognosis than E-cad+ ILC (p=0.005, Log-rank test). Figure 5 shows examples of E-cad IHC-staining. Table 7 shows that risk of death from breast cancer for ILC E-cad- was nearly two-fold (HR 1.96, 95 % CI 1.32–2.89) compared to IDC E-cad+. There was no clear difference in prognosis between IDC E-cad+, IDC E-cad- and ILC E-cad+. Adjustment for age, stage and time period did not influence the results.

Discussion

The main finding in this study of a cohort of breast cancer patients with long-term follow-up, was a significantly poorer prognosis for grade 2 ILC compared to grade 2 IDC. The prognosis for grade 2 ILC was comparable to that of grade 3 IDC. A similar pattern was observed when the analyses were restricted to tumours with positive prognostic marker profiles (ER+, HER2- and Ki67 low). Furthermore, E-cad expression appeared to be a favourable prognostic marker in ILC.

In the Nottingham grading system gland formation; nuclear atypia/pleomorphism and mitosis counts are considered [5]. However, because the morphological features of ILC differ from IDC, grade may have different prognostic significance[8, 21]. This is an important discussion because histopathological grade is one of several factors determining adjuvant therapy, whereas type is disregarded.

In agreement with others[1, 21, 22], there were few ILCs of grade 1 (7.8 %) and grade 3 (6.9 %) in this study, and the low numbers preclude survival analyses. Histopathological grading has been shown to be of independent prognostic value in ILC [23]. However, the implications of grading in ILC may differ from IDC and its value as a prognostic tool must be considered in this light, particularly when determining treatment strategies.

ER, HER2 and Ki67 are important prognostic and/or predictive markers. In this study, the proportion of ILCs with a favourable marker profile was higher compared to IDC, implying a better prognosis for ILC. However, even when restricting analyses to cases with favourable marker profiles, a significantly poorer prognosis was found in ILC compared to IDC. HER2+ cases in ILC were few (Table 2), thus limiting its utility as a prognostic marker in ILC. Better prognostic markers for ILC are required.

In this study, E-cad+ grade 2 ILC was prognostically comparable to grade 2 IDC (both E-cad+ and E-cad-). E-cad- ILC had a poorer prognosis. Identification of patients with ILC of expected poor prognosis may have implications when determining adjuvant therapy. If the prognostic utility of E-cad for ILC is confirmed in future studies and robust guidelines for interpretation of E-cad IHC are developed[14, 15], this could extend the use of a well-known marker for the benefit of a substantial proportion of breast cancer patients.

Loss of E-cad expression is shown to promote invasion and metastasis of epithelial cancers including breast cancer [24]. E-cad may be involved in other cellular processes of importance as

a tumour suppressor gene[25]. Cell-to-cell adhesion involves cytoplasmic catenins and the actin cytoskeleton in addition to E-cad, and these mechanisms are complex[26]. Loss of tumour suppressor function and impaired cell-to-cell adhesion, both of which are in part dependant on E-cad, underline the importance of this molecule in breast cancer.

The proportion of E-cad+ ILC reported varies from none to 20 %[27-29]. In this study, where histopathological typing was based on morphology only, 23.3 % were E-cad+. No cases were revised according to histopathological type in light of E-cad-status. Mixed lobular and ductal carcinomas are not infrequent [3]. In this study mixed tumours were classified as ductal[27, 30, 31].

Molecular subtyping is based mainly on studies of IDC[32]. IDC is the most common histopathological type though type is rarely mentioned[33-35]. For other types the prognostic value of molecular subtyping remains uncertain. In this study, there were too few ILCs in the non-luminal and HER2 subtypes for reliable results. However, the differences in BCSS in the HER2 negative luminal subtypes between ILC and IDC are comparable to the results of the biomarker analyses. The results considered together confirm that histopathological type has an independent impact in prognostication of ILC.

The main strength of this study is the historic nature of the patient cohort enabling complete long-term follow-up. The vast majority of women in this study developed breast cancer in an era prior to the use of hormonal contraception, menopausal hormonal therapy (MHT) and mammography screening, and did not qualify for new therapies as they were introduced thus enabling insight into the near-natural course of this disease. A drawback is the relative high age of the women and should be considered when interpreting the results. Others have shown better [36], similar [2, 37] or poorer [38, 39] prognosis for ILC compared to IDC . Differences in patient populations, follow-up and adjuvant therapy may explain these inconsistencies. Some studies have shown increased risk of ILC when using MHT[40-42]. It is unclear whether there

are differences in prognosis between MHT-associated ILC and ILC in non-users[43]. The majority of cancers in the present study were diagnosed in a time period or at an age when MHT was rarely used.

In this study, 99 of 116 ILCs were histopathological grade 2. The numbers of grade 1 and 3 were low and this can be attributed to the morphological features of ILC. This impairs grading as a prognostic tool in ILC. Similarly, the prognostic value of HER2 in ILC may be limited due to the low number of ILCs expressing HER2. However, grade 2 ILC had a consistently poorer prognosis when compared to grade 2 IDC, and the differences were also apparent when the analyses included only tumours with presumed favourable biomarkers. Due to the low number of lobular tumours in our study, we did not have sufficient statistical power to investigate the prognostic value of an unfavourable biomarker profile within lobular cancers. The present study supports the claim that lobular lesions are a distinct family of neoplastic lesions in the breast[12]. The role of E-cad in ILC may not only be in the determination of histopathological type, it may also be more useful than grade in prognostication and in the determination of treatment.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MJE contributed to the conception and design of the study, interpretation of the IHC, carried out statistical analyses, interpretation of the results and drafted the manuscript. SO participated in the statistical analyses and reviewed the manuscript. OAH carried out revision of cases for histopathological type and grade and reviewed the manuscript. LJV reviewed the manuscript. AMB contributed to conception and design of the study, carried out revision of

cases for histopathological type and grade, interpretation of the IHC, interpretation and analyses of the data, and draft and review of the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The study has received financial support from the Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology, The Research Council of Norway and the Cancer Fund, St. Olav's Hospital, Trondheim University Hospital, Norway.

The authors thank the Department of Pathology and Medical Genetics, St. Olav's Hospital for making the archives available for the study, the Cancer Registry of Norway for providing the patient data and senior biomedical scientist Borgny Ytterhus for her invaluable work in the laboratory.

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Table 1 Summary of patient and tumour characteristics.

Patient and tumour characteristics	**Ductal**	**Lobular**	**Total**
Number (%)	611 (84.0)	116 (16.0)	727 (100.0)
Number of breast cancer deaths (%)	246 (40.3)	51 (44.0)	297 (40.9)
Mean age at diagnosis (SD)	71.3 (10.7)	73.3 (9.1)	71.7 (10.5)
Median years of follow-up after diagnosis (IQR)	7.2 (10.6)	4.8 (7.9)	6.8 (10.4)
Tumour grade (%)			
1	61 (10.0)	9 (7.8)	70 (9.6)
2	317 (51.9)	99 (85.3)	416 (57.2)
3	233 (38.1)	8 (6.9)	241 (33.2)
Tumour size (%)			
≤ 2	182 (29.8)	20 (17.2)	202 (27.8)
>2, ≤ 5	221 (36.2)	43 (37.1)	264 (36.3)
>5	46 (7.5)	19 (16.4)	65 (8.9)
Uncertain	162 (26.1)	34 (29.3)	196 (27.0)
Lymph node status			
No metastasis	234 (38.3)	45 (38.8)	279 (38.4)
Metastasis detected	236 (38.6)	38 (32.8)	274 (37.7)
Not examined for metastasis	141 (23.1)	33 (28.4)	174 (23.9)
Stage at diagnosis			
Stage I	294 (48.1)	52 (44.8)	346 (47.6)
Stage II	246 (40.3)	49 (42.2)	295 (40.6)
Stage III	37 (6.1)	11 (9.5)	48 (6.6)
Stage IV	29 (4.8)	4 (3.5)	33 (4.5)
Stage uncertain	5 (0.8)	0	5 (0.7)
Molecular subtypes (%)			
Luminal A	290 (47.5)	63 (54.3)	353 (48.6)
Luminal B (HER2-)	170 (27.8)	33 (28.5)	203 (27.9)
Luminal B (HER2+)	54 (8.8)	6 (5.2)	60 (8.3)
HER2 type	49 (8.0)	1 (0.9)	50 (6.9)
Five negative phenotype	13 (2.1)	11 (9.5)	24 (3.3)
Basal phenotype	35 (5.7)	2 (1.7)	37 (5.1)

SD standard deviation, IQR interquartile range

Table 2. Summary of breast cancer therapies for all cases.

	Invasive ductal carcinoma n=611 (%)	**Invasive lobular carcinoma n=116 (%)**	**Total n=727 (%)**
Mastectomy	524 (85.8)	94 (81.0)	618 (85.0)
Breast conserving therapy	61 (10.0)	12 (10.4)	73 (10.0)
Only biopsy, no surgical treatment	26 (4.3)	10 (8.6)	36 (5.0)

Axillary surgery (clearance or sentinel node)	461 (75.5)	81 (69.9)	542 (74.6)
Hormone therapy*	134 (26.2**)	31 (30.4**)	165 (26.9**)
Trastuzumab	0	0	0
Chemotherapy	Unknown	Unknown	Unknown
Radiation	Unknown	Unknown	Unknown

* Estimated according to guidelines at diagnosis. ** % of the hormone receptor positive cases.

Table 3 Results of immunohistochemical and *in situ* hybridisation markers

	Ductal (n=611)	**Lobular (n=116)**	**Total (n=727)**
ER+	511 (83.6)	102 (87.9)	613 (84.3)
ER-	98 (16.0)	14 (12.1)	112 (15.4)
Not possible to interpret	2 (0.3)	0	2 (0.3)
PR+	364 (59.6)	58 (50.0)	422 (58.1)
PR-	246 (40.3)	58 (50.0)	304 (41.8)
Not possible to interpret	1 (0.2)	0	1 (0.1)
HER2+	103 (16.9)	7 (6.0)	110 (15.1)
HER2-	508 (83.1)	109 (94.0)	617 (84.9)
Ki67 high	280 (45.8)	39 (33.6)	319 (43.9)
Ki67low	330 (54.0)	77 (66.4)	407 (56.0)
Not possible to interpret	1 (0.2)	0	1 (0.1)
CK5+	120 (19.6)	4 (3.5)	124 (17.1)
CK5-	491 (80.4)	112 (96.6)	603 (82.9)
EGFR+	41 (6.7)	3 (2.6)	44 (6.1)
EGFR-	570 (93.3)	113 (97.4)	683 (93.9)
E-cad+	523 (85.6)	27 (23.3)	550 (75.7)
E-cad-	69 (11.3)	86 (74.1)	155 (21.3)
Not possible to interpret	19 (3.1)	3 (2.6)	22 (3.0)

Table 4. Risk of death from breast cancer. Invasive lobular carcinoma grade 2 compared to invasive ductal carcinoma grades 1, 2 and 3.

Tumour characteristics	Number of cases	Deaths from breast cancer	HR	95 % CI Unadjusted	HR	95 % CI Adjusted for age	HR	95 % CI Adjusted for stage	HR	95 % CI Adjusted for time period of diagnosis (10-year intervals)
Lobular grade 2	99	42	1.00		1.00		1.00		1.00	
Ductal grade 1	61	17	0.43	0.24 – 0.75	0.47	0.27 – 0.84	0.49	0.28 – 0.87	0.40	0.23 – 0.71
Ductal grade 2	317	114	0.66	0.46 – 0.94	0.67	0.47 – 0.95	0.59	0.41 – 0.85	0.66	0.46 – 0.94
Ductal grade 3	233	115	1.10	0.77 – 1.56	1.13	0.79 – 1.61	1.10	0.77 – 1.57	1.03	0.72 – 1.47
	710	297								

HR Hazard ratio. CI Confidence interval.

Table 5. Risk of death from invasive lobular grade 2 compared to invasive ductal carcinoma grade 2.

Tumour characteristics	Number of cases	Deaths from breast cancer	HR	95 % CI Unadjusted	HR	95 % CI Adjusted for age	HR	95 % CI Adjusted for stage	HR	95 % CI Adjusted for time period of diagnosis (10-year intervals)
ER positive										
Ductal	297	100	1.00		1.00		1.00		1.00	
Lobular	88	37	1.71	1.17 – 2.50	1.68	1.14 – 2.47	1.97	1.33 – 2.91	1.82	1.24 – 2.68
	385	137								
Ki67 low										
Ductal	224	71	1.00		1.00		1.00		1.00	
Lobular	70	30	2.01	1.31 – 3.01	1.95	1.26 – 3.03	2.20	1.42 – 3.43	2.03	1.31 – 3-14
	294	101								
HER2 negative										
Ductal	287	97	1.00		1.00		1.00		1.00	
Lobular	93	39	1.76	1.21 – 2.56	1.74	1.19 – 2.55	1.98	1.30 – 2.90	1.78	1.22 – 2.60
	380	136								
ER positive, Ki67 low and HER2 negative										

Ductal	201	61	1.00		1.00		1.00		1.00			
Lobular	56	24	2.16	1.34 – 3.49	2.04	1.25 – 3.34	2.45	1.50 – 4.01	2.31	1.42 – 3.76		
	257	85										

HR Hazard ratio. CI Confidence interval.

Table 6. Risk of death from invasive lobular carcinoma grade 2 and invasive ductal carcinoma grade 2 according to Luminal A and Luminal B (HER2-) subtypes.

	Number of cases	Deaths from breast cancer	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI
			Unadjusted		Adjusted for age		Adjusted for stage		Adjusted for time period of diagnosis (10-year intervals)	
Ductal Luminal A	203	62	1.00		1.00		1.00		1.00	
Ductal Luminal B (HER2-)	74	29	0.98	0.95 – 1.48	0.99	0.96 – 1.55	1.09	1.06 – 1.70	1.36	0.87 – 2.12
Lobular Luminal A	56	24	1.58	1.31 – 2.13	1.55	1.28 – 2.08	1.67	1.38 – 2.50	2.21	1.36 – 3.57
Lobular Luminal B (HER2-)	26	10	1.01	0.91 – 1.71	0.92	0.92 – 1.81	1.07	1.07 – 2.10	1.74	0.88 – 3.41
	359	125								

HR Hazard ratio. CI Confidence interval.

Table 7. Risk of death from invasive lobular carcinoma grade 2 and invasive ductal carcinoma grade 2 according to E-cadherin status.

	Number of cases	Deaths from breast cancer	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI
			Unadjusted		Adjusted for age		Adjusted for stage		Adjusted for time period of diagnosis (10-year intervals)	
Ductal, **E-cad positive**	260	94	1.00		1.00		1.00		1.00	
Ductal, **E-cad negative**	46	16	1.03	0.61 – 1.75	1.00	0.59 – 1.71	1.00	0.68 – 2.00	1.03	0.60 – 1.76
Lobular, **E-cad positive**	24	7	0.84	0.39 – 1.81	0.80	0.40 – 1.88	0.87	0.40 – 1.89	0.83	0.38 – 1.79
Lobular, **E-cad negative**	74	35	1.96	1.32 – 2.89	1.86	1.27 – 2.80	2.30	1.54 – 3.44	2.03	1.36 – 3.01
	404	152								

HR Hazard ratio. CI Confidence interval.

Figure legends

Figure 1: Classification algorithm for molecular subtyping [17].

Figure 2: Breast cancer specific survival for invasive lobular carcinoma grade 2 compared to ductal carcinoma grades 1, 2 and 3. P-value from log-rank test of differences in BCSS was 0.01.

Figure 3: Breast cancer specific survival for invasive lobular and ductal carcinoma grade 2 according to Luminal A and Luminal B (HER2-) subtypes. P-value from log-rank test of differences in BCSS was 0.02.

Figure 4: Breast cancer specific survival for for invasive lobular and ductal carcinoma grade 2 according to E-cadherin status. P-value from log-rank test of differences in BCSS was 0.005.

Figure 5: Invasive lobular carcinoma(ILC): **A**. ILC HES 400x. **B**. Same case as A. Positive membrane staining for E-cadherin. 400x. **C**. ILC HES 400x. **D**. Same case as C. No membrane staining for E-cadherin. 400x