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# BMJ Open

## The presence of axillary lymph node metastases at the time of definitive breast cancer surgery predicts shorter survival in metastatic disease

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3 The presence of axillary lymph node metastases at the time of definitive breast cancer surgery  
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5 predicts shorter survival in metastatic disease  
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56 Keywords: estrogen receptor; metastatic breast cancer; metastasis; nodal status; prognosis  
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## Abstract

**Background:** Although novel early breast cancer prognostic factors are being continuously discovered, only rare factors predicting survival in metastatic breast cancer have been validated. The prognostic role of early breast cancer prognostic factors in metastatic disease also remains mostly unclear.

**Methods:** We prospectively followed 594 women with early breast cancer. Sixty-one of these patients developed distant metastases during the follow-up, and their primary tumor properties, such as tumor size, nodal status, estrogen (ER) and progesterone receptor expression, grade, proliferation rate, histopathological subtype and breast cancer subtype were analyzed as potential prognostic factors for metastatic disease.

**Results:** In multivariate analysis, the presence of lymph node metastases at the time of early breast cancer surgery (hazard ratio (HR), 2.17; 95% confidence interval (CI), 1.09-4.31;  $p = 0.027$ ) and ER status (negative versus positive, HR, 2.16; 95% CI, 1.14-4.10;  $p = 0.018$ ) were significant predictors of survival in metastatic disease.

**Conclusions:** In conclusion, these results confirm ER status as a primary prognostic factor in metastatic breast cancer. Furthermore, it also suggests that the presence of initial lymph node metastases could serve as a prognostic factor in recurrent breast cancer.

## Article summary

### Strengths and limitations of this study

- We used a contemporary, prospective breast cancer cohort treated to determine whether primary breast cancer prognostic factors could also predict outcomes in recurrent metastatic breast cancer.

- The presence of local lymph node metastasis at the time of early breast cancer surgery predicted short survival in subsequent metastatic breast cancer.
- Our results also support previous results of ER negativity in primary breast cancer as an adverse prognostic factor in disease recurrence.
- If the results are validated, patients with lymph node metastasis at the time of early breast cancer surgery or patients with ER negative breast cancer should be considered for more aggressive first-line metastatic breast cancer therapy.
- The results may not be suitable for generalizing to *de novo* metastatic breast cancers, which have a different natural course from recurrent breast cancers.

## Introduction

Breast cancer is by far the most common and deadliest cancer affecting women worldwide [1]. In contrast to considerably prolonged early breast cancer prognosis during the last decade, which is mainly due to the optimization of adjuvant therapies, the prognosis of patients with metastatic breast cancer has hardly been prolonged, and the current median of overall survival is approximately 36 months [2-5].

The presence of nodal involvement is the strongest predictor of outcomes for early breast cancer [6]. The clinical behavior of metastatic breast cancer still varies greatly, and it is difficult to predict. The best validated prognostic factors in metastatic breast cancer include clinical factors, such as long relapse-free intervals, the absence of brain metastases or visceral metastases and the presence of estrogen receptor (ER), which also serves as an essential predictive factor in metastatic settings [7-12]. *De novo* metastatic breast cancers also have better prognosis than recurrent breast cancer [13, 14]. The possibility of using other characteristics of primary breast cancer, such as primary

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3 tumor size and axillary lymph node status as prognostic factors in metastatic breast cancer is still  
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5 being discussed; however, this approach has seldom been studied in modern prospective cohorts.  
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10 Using a large prospective breast cancer cohort treated with modern treatment modalities, we aimed  
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12 to determine whether primary breast cancer prognostic factors, such as tumor size, nodal status, ER  
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14 and progesterone receptor (PR) expression, differentiation, proliferation rate or breast cancer  
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16 subtype could also predict outcomes in recurrent metastatic breast cancer.  
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## 21 **Materials and methods**

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27 The original patient material was from a prospective dataset collected in Oulu University Hospital  
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29 from 2003–2013. The dataset consisted of 594 patients with early invasive breast cancer diagnosed  
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31 and treated in Oulu University Hospital, Finland. Patients with previous breast cancer or distant  
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33 metastases at the time of diagnosis were excluded from the cohort. During the follow-up, 61 women  
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35 displayed distant metastases, and the outcomes of these patients were reported in this study.  
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43 Tumors were classed into five intrinsic subtypes according to European Society for Medical  
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45 Oncology Clinical Practice Guidelines on Breast Cancer [15]. Luminal A-like carcinomas  
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47 expressed ER and PR, showed Ki-67 expression in  $< 15\%$  of the cells, and did not overexpress  
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49 HER2. Luminal B-like (HER2-negative) carcinomas were ER-positive and HER2-negative. In  
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51 addition, they showed either Ki-67 expression in  $\geq 15\%$  of cells, or they were PR-negative. Luminal  
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53 B-like (HER2-positive) tumors expressed ER and overexpressed HER2. Triple-negative breast  
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55 carcinomas (TNBC) were defined as tumors with no ER, PR and HER2 expression. HER2-positive  
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3 (non-luminal) cases overexpressed HER2 without ER or PR positivity. The distribution between  
4 subtypes in the cohort is described in detail in Table 1.  
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11 The histopathology was evaluated according to current WHO classification and stage was assessed  
12 using TNM classification. The expressions of ER, PR and Ki-67 were studied using  
13 immunohistochemistry as previously described [16]. HER2 expression was studied using  
14 immunohistochemistry and chromogenic in situ hybridization (CISH) to confirm positive results. A  
15 positive result of six or more gene copies in CISH was considered HER2-positive [17].  
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#### 26 Ethical considerations

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30 The patients provided their written informed consent to participate the study. The study was  
31 approved by the Local Ethics Committee of the Ostrobothnia Hospital District (114/2011) and the  
32 National Supervisory Authority for Welfare and Health (D9580/05.01.00.06/2010). All studies were  
33 conducted in accordance with the principles of the Declaration of Helsinki and the guidelines for  
34 good clinical practice.  
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#### 44 Statistical analyses

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49 Statistical analysis was performed using IBM SPSS Statistics software, v. 25.0.0.0 for Mac (IBM  
50 Corporation, Armonk, NY, USA). Survival was analyzed with Kaplan-Meier curves and the log-  
51 rank tests. Survival in metastatic disease was calculated from the date when metastasis was first  
52 observed in imaging to the time of death. Multivariate analysis was conducted using Cox  
53 multivariate regression analysis. P-values less than 0.05 were considered significant.  
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Table 1. Primary tumor characteristics.

	N (%)
<b>Tumor size</b>	
T1	20 (32.8%)
T2	33 (54.1%)
T3	7 (11.5%)
T4	1 (1.6%)
<b>Nodal status</b>	
N0	16 (26.2%)
N1	22 (36.1%)
N2	15 (24.6%)
N3	8 (13.1%)
<b>Histopathology</b>	
Ductal	47 (77.0%)
Lobular	11 (18.0%)
Other	3 (4.9%)
<b>Histopathological grade</b>	
Grade 1	0 (0%)
Grade 2	25 (41.0%)
Grade 3	34 (55.7%)
Unknown	2 (3.3%)
<b>ER expression</b>	
Negative (0 %)	14 (23.0%)
Weak (1-9 %)	2 (3.3%)

Moderate (10-59 %)	6 (9.8%)
High (>59 %)	39 (63.9%)
<b>PR expression</b>	
Negative (0 %)	22 (36.1%)
Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
<b>HER2 status</b>	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
<b>Ki-67 expression</b>	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
<b>Focality</b>	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
<b>Subtype</b>	
Luminal A-like	13 (21.3%)
Luminal B-like (HER2-negative)	29 (47.5%)
Luminal B-like (HER2-positive)	5 (8.2%)
HER2-positive, non-luminal	3 (4.9%)
Triple-negative	10 (16.4%)

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Unknown	1 (1.6%)
<b>The first site of the distant metastasis</b>	
Bone only	17 (27.9%)
Lung only	9 (14.8%)
Liver only	5 (8.2%)
Other	6 (9.6%)
Multiple sites	24 (39.3%)

### Patient and public involvement

Patients or public were not involved in the design, conduct, reporting or dissemination of this study.

### Results

Sixty-one patients of the originally 94 women ultimately developed distant metastases during the follow-up. Of these, fifty patients died of breast cancer during the follow-up. The median follow-up of the patients during their metastatic breast cancer was 18.0 months (mean 30.2 months). The median follow-up time starting from the early breast cancer diagnosis was 72.0 months in patients who later developed metastases.

Patients with metastatic local lymph nodes at the time of definitive surgery displayed poorer survival outcomes for metastatic disease ( $p = 0.031$ ) (Figure 1). The Kaplan-Meier estimate for median survival in metastatic disease in lymph node-negative patients was 33.0 months, and in lymph node-positive patients, it was 19.0 months. Only N0 versus N1-3 classification was

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3 significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes ( $p =$   
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5 0.78) were detected.  
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10 Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary  
11 tumor ( $p = 0.011$ ), breast cancer subtype (determined from the initial surgical samples) ( $p =$   
12 0.0078), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors ( $p = 0.032$ )  
13 and grade I–II (versus grade III) primary tumors ( $p = 0.012$ ) were associated with better survival in  
14 metastatic disease in univariate analysis. Primary tumor size, PR or HER2 expression or age at  
15 disease onset were not associated with metastatic disease survival.  
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26 When assessed separately by different biological subtypes, initial lymph node metastases predicted  
27 worse prognosis only in the patients with the luminal A subtype in univariate analysis ( $p = 0.019$ ),  
28 but the small sample size of each subgroup limited the reliability of this analysis (data not shown).  
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35 In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis  
36 predicted poorer survival overall (HR, 2.17; 95% CI, 1.09–4.31;  $p = 0.027$ ) when tumor size (T1  
37 versus T2–4) (HR, 1.33; 95% CI, 0.71–2.47;  $p = 0.37$ ) and ER status (negative versus positive) (HR  
38 2.16; 95% CI 1.14–4.10;  $p = 0.018$ ) were included in the analysis. Breast cancer subtype, Ki-67  
39 expression or grade did not remain significant prognostic factors after multivariate analysis.  
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## 49 **Discussion**

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54 As the main observation, we report in this prospectively collected and contemporary cohort from a  
55 Finnish University Hospital that the presence of local lymph node metastasis at the time of early  
56 breast cancer surgery predicted short survival in subsequent metastatic breast cancer. Our results  
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3 also supported previous results of ER negativity in primary breast cancer as an adverse prognostic  
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5 factor for disease recurrence.  
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10 The most established prognostic factors of better outcome in metastatic breast cancer include ER  
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12 positivity, long disease-free interval (usually defined as at least 2 years), low number of metastatic  
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14 sites and bone-only localization of metastases [7-14, 18-20]. HER2 appears to no longer represent a  
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16 prognostic factor in the era of targeted treatments, and prognostic factors also slightly differ  
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18 between HER2-positive and HER2-negative patients [21, 22]. Emerging metastatic breast cancer  
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20 prognostic factors include circulating tumor cells, gene expression panels, circulating tumor  
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22 markers and miRNAs; however, they have not yet been sufficiently validated [23-26]. Whereas ER  
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24 status, a lengthy disease-free interval and metastatic load are established and obvious prognostic  
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26 factors for metastatic breast cancer, the presence of lymph node metastases at the time of initial  
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28 diagnosis has not been widely studied in metastatic breast cancer, although it is the strongest  
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30 prognostic factor in early breast cancer. In the current study, we concentrated solely on primary  
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32 breast cancer characteristics, and we did not assess other characteristics, such as disease-free  
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34 interval, metastasis load or metastasis location as prognostic factors.  
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42 Some previous studies with mostly retrospective cohort settings and outdated treatment modalities  
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44 have reported the initial nodal status as a prognostic factor in metastatic breast cancer, whereas  
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46 others have not found such an association [7, 27-29]. In the pioneer work of Clark et al., nodal  
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48 involvement at time of initial diagnosis was associated with shorter survival [12]. Another  
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50 retrospective single-institute study also concluded that lymph node involvement at primary  
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52 diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients  
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54 were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish  
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3 retrospective registry study suggested that the initial lymph node status should be part of the  
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5 prognostic index in recurrent metastatic breast cancer [31].  
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10 In our study, any metastasis found in local lymph nodes at the time of definitive surgery was  
11 associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median  
12 survival was prolonged from 19–33 months in patients without lymph node metastases at the time  
13 of initial diagnosis. Although lymph node metastases in general is associated with other factors of  
14 poor prognosis, our study suggested that this result was independent of tumor size and ER  
15 expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it  
16 can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent  
17 paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions  
18 suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for  
19 predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per*  
20 *se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually  
21 metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary  
22 lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these  
23 issues continues [36].  
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45 It has to be emphasized that all our patients had recurrent breast cancer, and our material did not  
46 include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the  
47 material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast  
48 cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless,  
49 the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer,  
50 and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9,  
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3 37]. Our study was based on a prospective cohort from a university clinic, and the patients were  
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5 treated with up-to-date surgical and oncological treatment modalities.  
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10 Our results confirmed that ER negativity in primary tumor was associated with short survival for  
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12 metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but  
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14 also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER  
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16 negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12,  
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18 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER  
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20 status is also a predictor of poor prognosis [38]. TNBC has the worst outcome of all subtypes in  
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22 metastatic breast cancer, a finding which was mirrored in our study [39].  
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28 Predicting the course of metastatic breast cancer is of primary importance in clinical practice;  
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30 however, its status as a highly heterogenous disease at both the inpatient and outpatient levels  
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32 makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer  
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34 guidelines recommend starting treatment with chemotherapy or even with a combination  
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36 chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing  
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38 ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic  
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40 breast cancer, such as initial nodal status, could be confirmed, these patients should receive more  
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42 aggressive first-line metastatic breast cancer therapy.  
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49 In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse  
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51 prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial  
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53 lymph node status may be a prognostic factor for metastatic disease course. Future studies should  
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55 also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute  
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57 number of metastatic lymph nodes, which were not addressed in our material. More research is also  
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3 clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or  
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5 whether they are purely an indicator of aggressive disease.  
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### 10 **Contributorship statement**

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14 All authors contributed to the study design and conception. AJu initiated the collection of the  
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16 prospective dataset. PK, AJā and NR were responsible for assessing statistical analyses. PK was a  
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18 major contributor in writing the manuscript. All authors provided comments on drafts of the  
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20 manuscript. All authors read and approved the final manuscript.  
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### 26 **Data sharing statement**

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30 No additional data available.  
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### 35 **Figure legends**

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40 Figure 1. Associations between primary tumor properties and survival in metastatic breast cancer.  
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42 In multivariate analysis, only ER expression and initial nodal status remained as significant factors.  
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### 51 **References**

- 52  
53  
54 1 Bray F, Ferlay J, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and  
55 mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.  
56  
57 2 Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO International Consensus Guidelines for  
58 Advanced Breast Cancer (ABC 4)dagger. *Ann Oncol* 2018;29:1634-57.  
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3 Cardoso F, Spence D, Mertz S, Corneliussen-James D, Sabelko K, Gralow J, et al. Global analysis of advanced/metastatic breast cancer: Decade report (2005-2015). *Breast* 2018;39:131-8.

4 Allemani C, Weir HK, Carreira H, et al. Global surveillance of cancer survival 1995-2009: analysis of individual data for 25,676,887 patients from 279 population-based registries in 67 countries (CONCORD-2). *Lancet* 2015;385:977-1010.

5 Weigelt B, Peterse JL, van 't Veer, LJ. Breast cancer metastasis: markers and models. *Nat Rev Cancer* 2005;5:591-602.

6 Early Breast Cancer Trialists' Collaborative Group, (EBCTCG), Peto R, Davies C, Godwin J, Gray R, Pan HC, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet* 2012;379:432-44.

7 Chang J, Clark GM, Allred DC, et al. Survival of patients with metastatic breast carcinoma: importance of prognostic markers of the primary tumor. *Cancer* 2003;97:545-53.

8 Regierer AC, Wolters R, Ufen M, et al. An internally and externally validated prognostic score for metastatic breast cancer: analysis of 2269 patients. *Ann Oncol* 2014;25:633-8.

9 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast cancer. *Ann Oncol* 2008;19:2012-9.

10 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-free interval is important. *Hum Pathol* 2017;70:70-6.

11 Stuart-Harris R, Shadbolt B, Palmqvist C, et al. The prognostic significance of single hormone receptor positive metastatic breast cancer: an analysis of three randomised phase III trials of aromatase inhibitors. *Breast* 2009;18:351-5.

12 Clark GM, Sledge GW, Jr, Osborne CK, et al. Survival from first recurrence: relative importance of prognostic factors in 1,015 breast cancer patients. *J Clin Oncol* 1987;5(1):55-61.

13 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-free interval is important. *Hum Pathol* 2017;70:70-6.

14 Lobbezoo DJA, van Kampen, RJW., Voogd AC, et al. Prognosis of metastatic breast cancer: are there differences between patients with de novo and recurrent metastatic breast cancer? *Br J Cancer* 2015;112:1445-51.

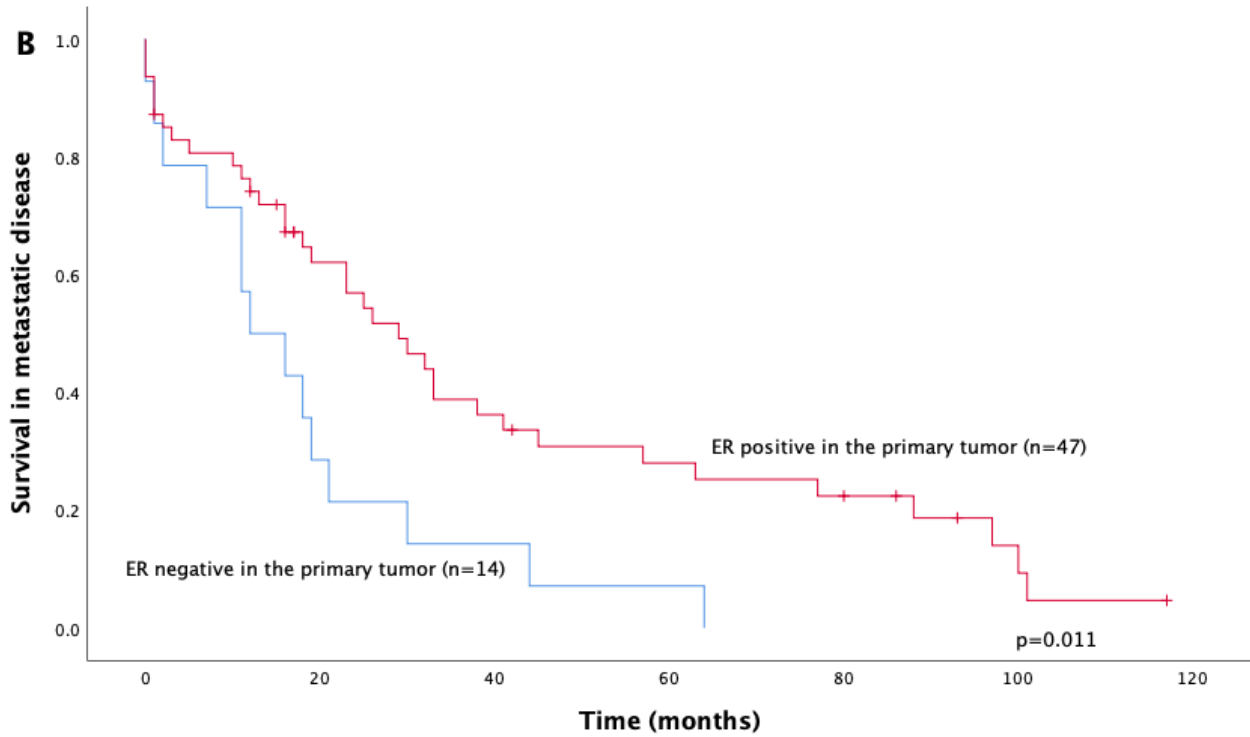
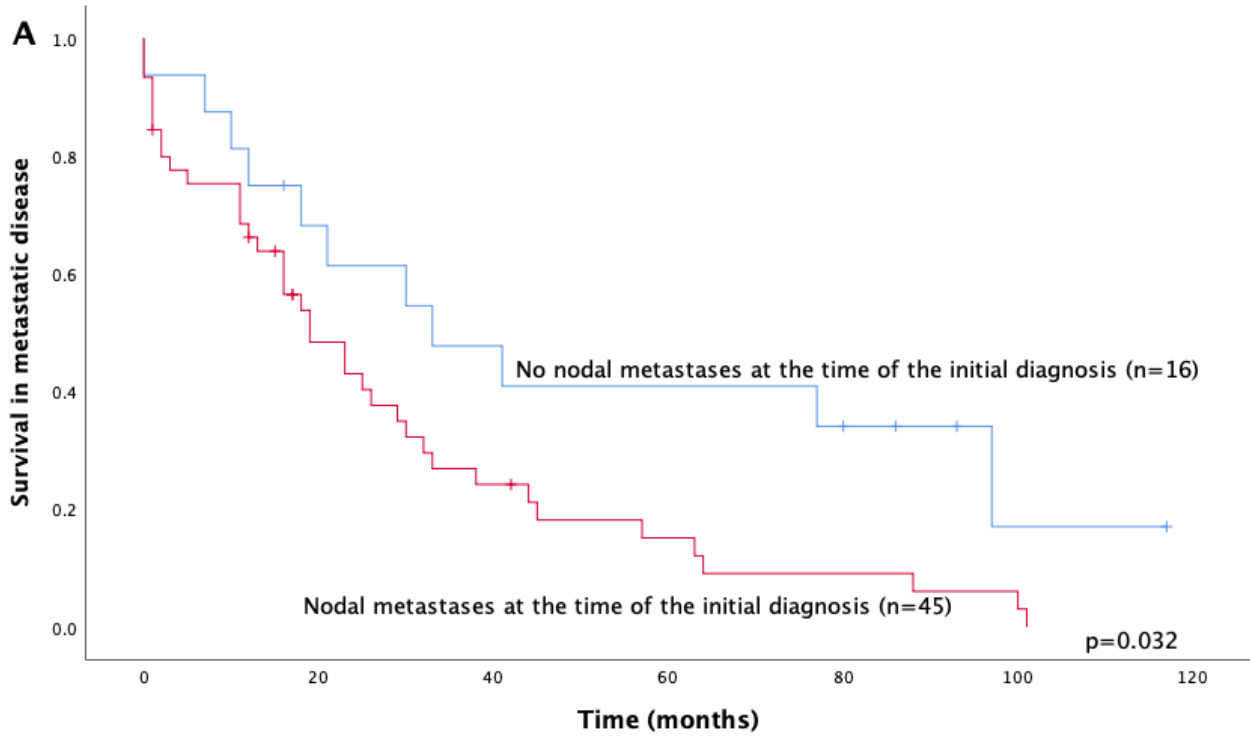
15 Senkus E, Kyriakides S, Ohno S, et al. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26 Suppl 5:8.

16 Karihtala P, Mäntyniemi A, Kang SW, et al. Peroxiredoxins in breast carcinoma. *Clin Cancer Res* 2003;9:3418-24.

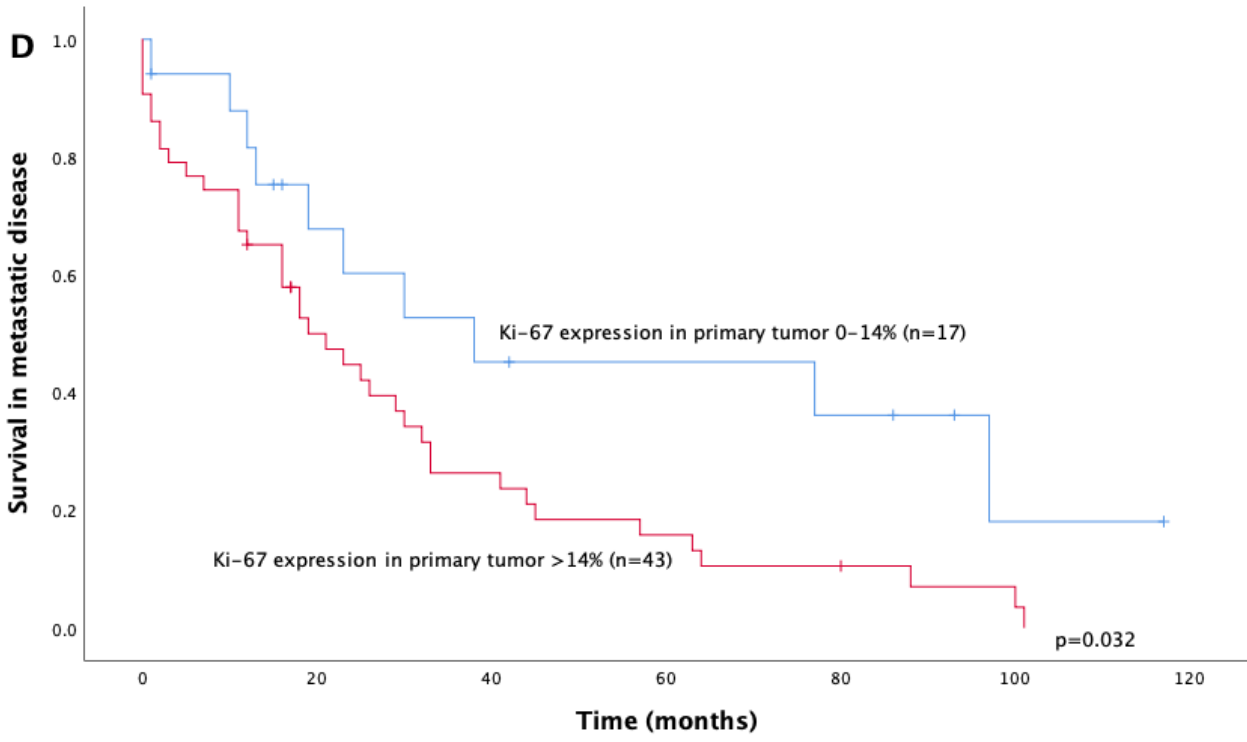
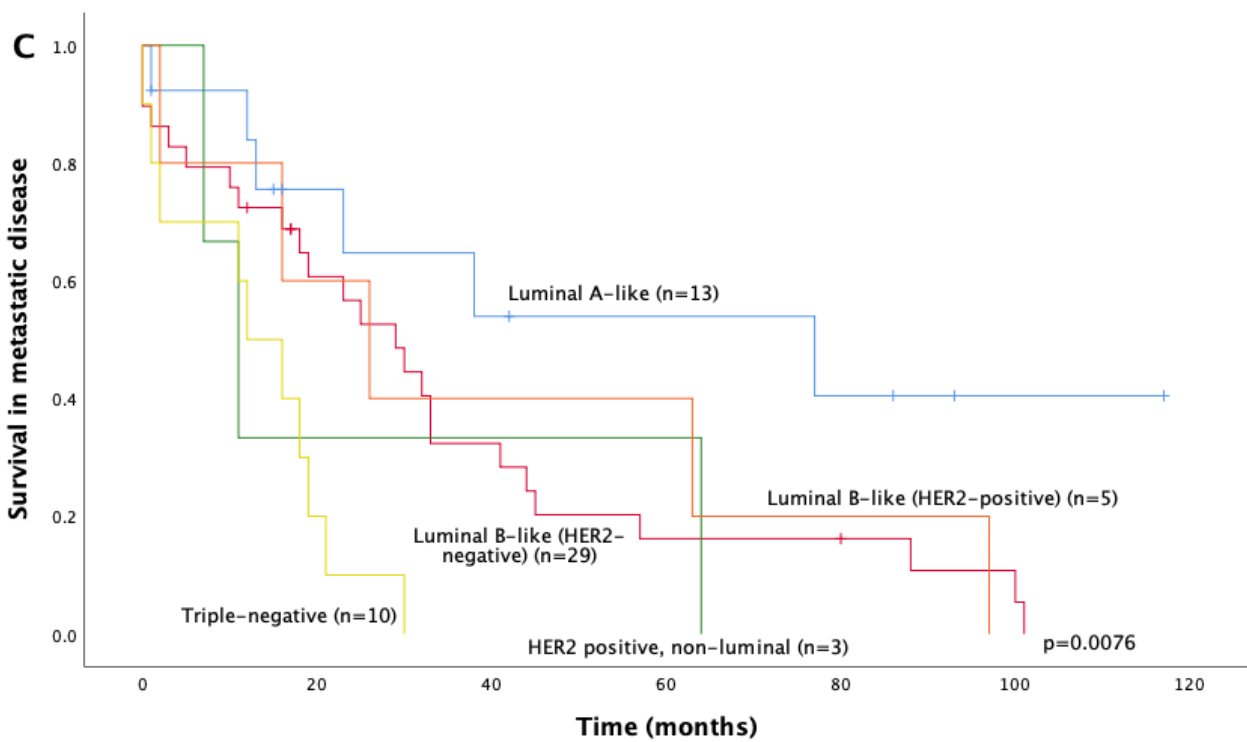
17 Isola J, Tanner M, Forsyth A, et al. Interlaboratory comparison of HER-2 oncogene amplification as detected by chromogenic and fluorescence in situ hybridization. *Clin Cancer Res* 2004;10:4793-8.

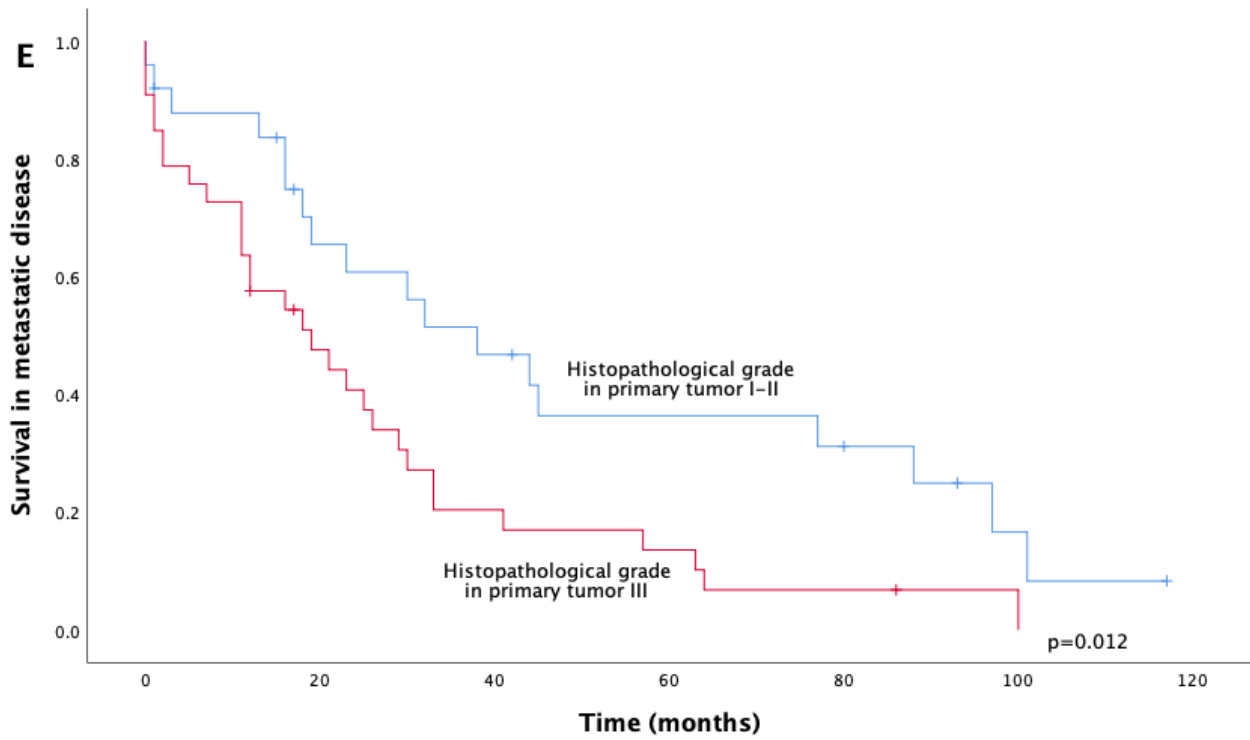
- 1  
2  
3 18 Yamamoto N, Watanabe T, Katsumata N, et al. Construction and validation of a practical  
4 prognostic index for patients with metastatic breast cancer. *J Clin Oncol* 1998;16:2401-8.  
5  
6  
7 19 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast  
8 cancer patients: analysis of deceased patients. *Rad Oncol J* 2013;31:222-7.  
9  
10 20 Khanfir A, Lahiani F, Bouzguenda R, et al. Prognostic factors and survival in metastatic breast  
11 cancer: A single institution experience. *Rep Pract Oncol Radiother* 2013;18:127-32.  
12  
13 21 Hopkins AM, Rowland A, McKinnon RA, et al. Predictors of Long-Term Disease Control and  
14 Survival for HER2-Positive Advanced Breast Cancer Patients Treated With Pertuzumab,  
15 Trastuzumab, and Docetaxel. *Front Oncol* 2019;9:789.  
16  
17  
18 22 Dawood S, Broglio K, Buzdar AU, et al. Prognosis of women with metastatic breast cancer by  
19 HER2 status and trastuzumab treatment: an institutional-based review. *J Clin Oncol* 2010;28:92-8.  
20  
21 23 King TA, Lyman JP, Gonen M, et al. Prognostic Impact of 21-Gene Recurrence Score in  
22 Patients With Stage IV Breast Cancer: TBCRC 013. *J Clin Oncol* 2016 Jul 10;34(20):2359-65.  
23  
24  
25 24 Prat A, Cheang MC, Galvan P, et al. Prognostic Value of Intrinsic Subtypes in Hormone  
26 Receptor-Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib.  
27 *JAMA Oncol* 2016;2:1287-94.  
28  
29  
30 25 Papadaki C, Stoupis G, Tsalikis L, et al. Circulating miRNAs as a marker of metastatic disease  
31 and prognostic factor in metastatic breast cancer. *Oncotarget* 2019;10:966-81.  
32  
33  
34 26 Van Poznak C, Somerfield MR, Bast RC, et al. Use of Biomarkers to Guide Decisions on  
35 Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical  
36 Oncology Clinical Practice Guideline. *J Clin Oncol* 2015;33(24):2695-704.  
37  
38 27 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast  
39 cancer. *Ann Oncol* 2008;19:2012-9.  
40  
41  
42 28 Insa A, Lluch A, Prosper F, et al. Prognostic factors predicting survival from first recurrence in  
43 patients with metastatic breast cancer: analysis of 439 patients. *Breast Cancer Res Treat*  
44 1999;56(1):67-78.  
45  
46  
47 29 Tsuji W, Teramukai S, Ueno M, et al. Prognostic factors for survival after first recurrence in  
48 breast cancer: a retrospective analysis of 252 recurrent cases at a single institution. *Breast Cancer*  
49 2014;21:86-95.  
50  
51  
52 30 Rack B, Janni W, Gerber B, et al. Patients with recurrent breast cancer: does the primary axillary  
53 lymph node status predict more aggressive tumor progression? *Breast Cancer Res Treat*  
54 2003;82(2):83-92.  
55  
56  
57 31 Puente J, Lopez-Tarruella S, Ruiz A, et al. Practical prognostic index for patients with metastatic  
58 recurrent breast cancer: retrospective analysis of 2,322 patients from the GEICAM Spanish El  
59 Alamo Register. *Breast Cancer Res Treat* 2010;122:591-600.  
60

- 1  
2  
3 32 Gibert-Ramos A, Lopez C, Bosch R, et al. Immune response profile of primary tumour, sentinel  
4 and non-sentinel axillary lymph nodes related to metastasis in breast cancer: an  
5 immunohistochemical point of view. *Histochem Cell Biol* 2019;152:177-93.  
6  
7  
8 33 Ullah I, Karthik G, Alkodsai A, et al. Evolutionary history of metastatic breast cancer reveals  
9 minimal seeding from axillary lymph nodes. *J Clin Invest* 2018;128:1355-70.  
10  
11 34 Klein CA. Selection and adaptation during metastatic cancer progression. *Nature* 2013 Sep  
12 19;501:365-72.  
13  
14 35 Engel J, Emeny RT, Hölzel D. Positive lymph nodes do not metastasize. *Cancer Metastasis Rev*  
15 2012;31:235-46.  
16  
17  
18 36 Barry P, Vatsiou A, Spiteri I, et al. The Spatiotemporal Evolution of Lymph Node Spread in  
19 Early Breast Cancer. *Clin Cancer Res* 2018;24:4763-70.  
20  
21 37 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast  
22 cancer patients: analysis of deceased patients. *Radiat Oncol J* 2013;31:222-7.  
23  
24  
25 38 Woo JW, Chung YR, Ahn S, et al. Changes in Biomarker Status in Metastatic Breast Cancer and  
26 Their Prognostic Value. *J Breast Cancer* 2019;22:439-52.  
27  
28  
29 39 Fietz T, Tesch H, Rauh J, et al. Palliative systemic therapy and overall survival of 1,395 patients  
30 with advanced breast cancer - Results from the prospective German TMK cohort study. *Breast*  
31 2017;34:122-30.  
32  
33 40 Januskeviciene I, Petrikaite V. Heterogeneity of breast cancer: The importance of interaction  
34 between different tumor cell populations. *Life Sci* 2019;239:117009.  
35  
36  
37 41 Breast Cancer [Internet].; 2019 [updated Sep 6,; cited Jan 12, 2019]. Available from:  
38  
39 [https://www.nccn.org/professionals/physician\\_gls/pdf/breast.pdf](https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf).  
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# BMJ Open

## Prognostic factors in metastatic breast cancer: a prospective single-center study

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3 Prognostic factors in metastatic breast cancer: a prospective single-center study  
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## Abstract

**Objectives:** Although novel early breast cancer prognostic factors are being continuously discovered, only rare factors predicting survival in metastatic breast cancer have been validated. The prognostic role of early breast cancer prognostic factors in metastatic disease also remains mostly unclear.

**Design and setting:** Prospective study in a Finnish University Hospital.

**Participants and outcomes:** 594 women with early breast cancer were originally followed. Sixty-one of these patients developed distant metastases during the follow-up, and their primary breast cancer properties, such as tumor size, nodal status, estrogen (ER) and progesterone receptor expression, grade, proliferation rate, histopathological subtype and breast cancer subtype were analyzed as potential prognostic factors for metastatic disease.

**Results:** In multivariate analysis, the presence of lymph node metastases at the time of early breast cancer surgery (hazard ratio (HR), 2.17; 95% confidence interval (CI), 1.09-4.31;  $p = 0.027$ ) and ER status (negative versus positive, HR, 2.16; 95% CI, 1.14-4.10;  $p = 0.018$ ) were significant predictors of survival in metastatic disease.

**Conclusions:** These results confirm ER status as a primary prognostic factor in metastatic breast cancer. Furthermore, it also suggests that the presence of initial lymph node metastases could serve as a prognostic factor in recurrent breast cancer.

## Article summary

### Strengths and limitations of this study

- This was a study of contemporary, prospective breast cancer cohort in a University Hospital with a relatively long follow-up.
- The material did not include patients with *de novo* metastatic breast cancer.

- The number of patients with metastatic breast cancer should have been larger to make more detailed subgroup analyses, regarding e.g. biological subgroups possible.

## Introduction

Breast cancer is by far the most common and deadliest cancer affecting women worldwide [1]. In contrast to considerably prolonged early breast cancer prognosis during the last decade, which is mainly due to the optimization of adjuvant therapies, the prognosis of patients with metastatic breast cancer has hardly been prolonged, and the current median of overall survival is approximately 36 months [2-5].

The presence of nodal involvement is the strongest predictor of outcomes for early breast cancer [6]. The clinical behavior of metastatic breast cancer still varies greatly, and it is difficult to predict. The best validated prognostic factors in metastatic breast cancer include clinical factors, such as long relapse-free intervals, the absence of brain metastases or visceral metastases and the presence of estrogen receptor (ER), which also serves as an essential predictive factor in metastatic settings [7-12]. *De novo* metastatic breast cancers also have better prognosis than recurrent breast cancer [13, 14]. The possibility of using other characteristics of primary breast cancer, such as primary tumor size and axillary lymph node status as prognostic factors in metastatic breast cancer is still being discussed; however, this approach has seldom been studied in modern prospective cohorts.

Using a large prospective breast cancer cohort treated with modern treatment modalities, we aimed to determine whether primary breast cancer prognostic factors, such as tumor size, nodal status, ER and progesterone receptor (PR) expression, differentiation, proliferation rate or breast cancer subtype could also predict outcomes in recurrent metastatic breast cancer.

## Materials and methods

The original patient material was from a prospective dataset collected in Oulu University Hospital from 2003–2013. The dataset consisted of 594 patients with early invasive breast cancer diagnosed and treated in Oulu University Hospital, Finland. Surgery to the primary tumor was carried out according to the guidelines of Finnish Breast Cancer Group. The dataset did not include information of the possible neoadjuvant chemotherapy, which was nevertheless very rarely administered during the study period. Patients with previous breast cancer or distant metastases at the time of diagnosis were excluded from the cohort (Figure 1). During the follow-up, 61 women displayed distant metastases, and the outcomes of these patients were reported in this study.

Tumors were classed into five intrinsic subtypes according to European Society for Medical Oncology Clinical Practice Guidelines on Breast Cancer [15]. Luminal A-like carcinomas expressed ER and PR, showed Ki-67 expression in  $< 15\%$  of the cells, and did not overexpress HER2. Luminal B-like (HER2-negative) carcinomas were ER-positive and HER2-negative. In addition, they showed either Ki-67 expression in  $\geq 15\%$  of cells, or they were PR-negative. Luminal B-like (HER2-positive) tumors expressed ER and overexpressed HER2. Triple-negative breast carcinomas (TNBC) were defined as tumors with no ER, PR and HER2 expression. HER2-positive (non-luminal) cases overexpressed HER2 without ER or PR positivity. The distribution between subtypes in the cohort is described in detail in Table 1.

The histopathology was evaluated according to current WHO classification and stage was assessed using TNM classification. The expressions of ER, PR and Ki-67 were studied using

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3 immunohistochemistry as previously described [16]. HER2 expression was studied using  
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5 immunohistochemistry and chromogenic in situ hybridization (CISH) to confirm positive results. A  
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7 positive result of six or more gene copies in CISH was considered HER2-positive [17] .  
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## 10 11 12 Ethical considerations

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17 The patients provided their written informed consent to participate the study. The study was  
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19 approved by the Local Ethics Committee of the Ostrobothnia Hospital District (114/2011) and the  
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21 National Supervisory Authority for Welfare and Health (D9580/05.01.00.06/2010). All studies were  
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23 conducted in accordance with the principles of the Declaration of Helsinki and the guidelines for  
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25 good clinical practice.  
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## 30 31 Statistical analyses

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35 Statistical analysis was performed using IBM SPSS Statistics software, v. 25.0.0.0 for Mac (IBM  
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37 Corporation, Armonk, NY, USA). Survival was analyzed with Kaplan-Meier curves and the log-  
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39 rank tests. Correction was not made for multiple comparisons. Survival in metastatic disease was  
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41 calculated from the date when metastasis was first observed in imaging to the time of death.  
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44 Multivariate analysis was conducted using Cox multivariate regression analysis. P-values less than  
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46 0.05 were considered significant.  
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51 Table 1. Primary tumor characteristics.

	N (%)
<b>Tumor size</b>	
T1	20 (32.8%)

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T2	33 (54.1%)
T3	7 (11.5%)
T4	1 (1.6%)
<b>Nodal status</b>	
N0	16 (26.2%)
N1	22 (36.1%)
N2	15 (24.6%)
N3	8 (13.1%)
<b>Histopathology</b>	
Ductal	47 (77.0%)
Lobular	11 (18.0%)
Other	3 (4.9%)
<b>Histopathological grade</b>	
Grade 1	0 (0%)
Grade 2	25 (41.0%)
Grade 3	34 (55.7%)
Unknown	2 (3.3%)
<b>ER expression</b>	
Negative (0 %)	14 (23.0%)
Weak (1-9 %)	2 (3.3%)
Moderate (10-59 %)	6 (9.8%)
High (>59 %)	39 (63.9%)
<b>PR expression</b>	
Negative (0 %)	22 (36.1%)

Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
<b>HER2 status</b>	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
<b>Ki-67 expression</b>	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
<b>Focality</b>	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
<b>Subtype</b>	
Luminal A-like	13 (21.3%)
Luminal B-like (HER2-negative)	29 (47.5%)
Luminal B-like (HER2-positive)	5 (8.2%)
HER2-positive, non-luminal	3 (4.9%)
Triple-negative	10 (16.4%)
Unknown	1 (1.6%)
<b>The first site of the distant metastasis</b>	
Bone only	17 (27.9%)
Lung only	9 (14.8%)

Liver only	5 (8.2%)
Other	6 (9.6%)
Multiple sites	24 (39.3%)

## Patient and public involvement

Patients or public were not involved in the design, conduct, reporting or dissemination of this study.

## Results

Sixty-one patients of the originally 594 women ultimately developed distant metastases during the follow-up. Of these, fifty patients died of breast cancer during the follow-up. The median disease-free interval was 39.0 months in the patients who had distant metastases. The median follow-up time starting from the early breast cancer diagnosis was 72.0 months in patients who later developed metastases.

The median follow-up of the patients during their metastatic breast cancer was 18.0 months (mean 30.2 months). The Kaplan-Meier estimate for median survival of the patients with metastatic breast cancer was 77.0 months in those with luminal A-like breast cancers, 29.0 months in those with luminal B-like (HER2-negative) disease and 11.0, 26.0 and 12.0 months in those with HER2-positive, non-luminal, luminal B-like (HER2-positive) and TNBC subtype, respectively.

Patients with metastatic local lymph nodes at the time of definitive surgery displayed poorer survival outcomes for metastatic disease ( $p = 0.031$ ) (Figure 2). The Kaplan-Meier estimate for median survival in metastatic disease in lymph node-negative patients was 33.0 months, and in



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3 lymph node-positive patients, it was 19.0 months. Only N0 versus N1-3 classification was  
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5 significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes ( $p =$   
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7 0.78) were detected.  
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12 Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary  
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14 tumor ( $p = 0.011$ ), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors ( $p$   
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16 = 0.032) and grade I–II (versus grade III) primary tumors ( $p = 0.012$ ) were associated with better  
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18 survival in metastatic disease in univariate analysis. Breast cancer subtype (determined from the  
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20 initial surgical samples) also predicted survival with metastatic breast cancer ( $p = 0.0078$ ) (Figure  
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22 2C). Also, the patients with luminal A-like breast cancer had significantly prolonged survival, when  
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24 compared to all other subtypes ( $p = 0.017$ ). Primary tumor size, PR or HER2 expression, the site of  
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26 the first metastasis in bone versus elsewhere, disease-free interval ( $\leq 24$  months versus  $> 24$   
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28 months) or age at disease onset were not associated with metastatic disease survival.  
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35 When assessed separately by different biological subtypes, initial lymph node metastases predicted  
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37 worse prognosis only in the patients with the luminal A subtype in univariate analysis ( $p = 0.019$ ),  
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39 but the small sample size of each subgroup limited the reliability of this analysis (data not shown).  
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45 In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis  
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47 predicted poorer survival overall (HR, 2.17; 95% CI, 1.09-4.31;  $p = 0.027$ ) when tumor size (T1  
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49 versus T2-4) (HR, 1.33; 95% CI, 0.71-2.47;  $p = 0.37$ ) and ER status (negative versus positive) (HR  
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51 2.16; 95% CI 1.14-4.10;  $p = 0.018$ ) were included in the analysis. Breast cancer subtype, Ki-67  
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53 expression or grade did not remain significant prognostic factors after multivariate analysis.  
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## 57 58 **Discussion** 59 60

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5 As the main observation, we report in this prospectively collected and contemporary cohort from a  
6 Finnish University Hospital that the presence of local lymph node metastasis at the time of early  
7 breast cancer surgery predicted short survival in subsequent metastatic breast cancer. Our results  
8 also supported previous results of ER negativity in primary breast cancer as an adverse prognostic  
9 factor for disease recurrence.  
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19 The most established prognostic factors of better outcome in metastatic breast cancer include ER  
20 positivity, long disease-free interval (usually defined as at least 2 years), low number of metastatic  
21 sites and bone-only localization of metastases [7-14, 18-20]. HER2 appears to no longer represent a  
22 prognostic factor in the era of targeted treatments, and prognostic factors also slightly differ  
23 between HER2-positive and HER2-negative patients [21, 22]. Emerging metastatic breast cancer  
24 prognostic factors include circulating tumor cells, gene expression panels, circulating tumor  
25 markers and miRNAs; however, they have not yet been sufficiently validated [23-26]. Whereas ER  
26 status, a lengthy disease-free interval and metastatic load are established and obvious prognostic  
27 factors for metastatic breast cancer, the presence of lymph node metastases at the time of initial  
28 diagnosis has not been widely studied in metastatic breast cancer, although it is the strongest  
29 prognostic factor in early breast cancer. In the current study, we concentrated solely on primary  
30 breast cancer characteristics, and we did not assess other characteristics, such as disease-free  
31 interval, metastasis load or metastasis location as prognostic factors.  
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51 Some previous studies with mostly retrospective cohort settings and outdated treatment modalities  
52 have reported the initial nodal status as a prognostic factor in metastatic breast cancer, whereas  
53 others have not found such an association [7, 27-29]. In the pioneer work of Clark et al., nodal  
54 involvement at time of initial diagnosis was associated with shorter survival [12]. Another  
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3 retrospective single-institute study also concluded that lymph node involvement at primary  
4 diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients  
5 were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish  
6 retrospective registry study suggested that the initial lymph node status should be part of the  
7 prognostic index in recurrent metastatic breast cancer [31]. In addition to considerable change in the  
8 oncological treatments of breast cancer during the last decades, also surgical techniques, especially  
9 axillary procedures have developed considerably. The current results from the prospective data with  
10 modern treatments thus support and confirm earlier results.  
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24 In our study, any metastasis found in local lymph nodes at the time of definitive surgery was  
25 associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median  
26 survival was prolonged from 19–33 months in patients without lymph node metastases at the time  
27 of initial diagnosis. Although lymph node metastases in general is associated with other factors of  
28 poor prognosis, our study suggested that this result was independent of tumor size and ER  
29 expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it  
30 can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent  
31 paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions  
32 suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for  
33 predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per*  
34 *se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually  
35 metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary  
36 lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these  
37 issues continues [36].  
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3 It has to be emphasized that all our patients had recurrent breast cancer, and our material did not  
4 include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the  
5 material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast  
6 cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless,  
7 the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer,  
8 and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9,  
9 37]. As an other limitation, we were unable to address the results separately in subgroups, for  
10 example according to biological subtypes, due to relatively low number of patients with metastatic  
11 breast cancer. On the other hand, our study was based on a prospective cohort from a university  
12 clinic, and the patients were treated with up-to-date surgical and oncological treatment modalities.  
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28 Our results confirmed that ER negativity in primary tumor was associated with short survival for  
29 metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but  
30 also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER  
31 negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12,  
32 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER  
33 status is also a predictor of poor prognosis [38]. Most previous studies have divided metastatic  
34 breast cancers only to three subgroups: ER/PR-positive, HER2-positive and TNBC. We used the  
35 widely recognized ESMO guidelines for subtyping our cases. Although the number of patients in  
36 each subgroup were rather limited, the patients with slowly proliferating, ER-responsive luminal A-  
37 like breast cancers still had significantly prolonged survival in metastatic breast cancer compared to  
38 other subtypes. TNBC has the worst outcome of all subtypes in metastatic breast cancer, a finding  
39 which was mirrored in our study [39].  
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3 Predicting the course of metastatic breast cancer is of primary importance in clinical practice;  
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5 however, its status as a highly heterogenous disease at both the inpatient and outpatient levels  
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7 makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer  
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9 guidelines recommend starting treatment with chemotherapy or even with a combination  
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11 chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing  
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13 ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic  
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15 breast cancer, such as initial nodal status, could be confirmed, these patients should receive more  
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17 aggressive first-line metastatic breast cancer therapy.  
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24 In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse  
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26 prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial  
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28 lymph node status may be a prognostic factor for metastatic disease course. Future studies should  
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30 also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute  
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32 number of metastatic lymph nodes, which were not addressed in our material. More research is also  
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34 clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or  
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36 whether they are purely an indicator of aggressive disease.  
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### 42 **Contributorship statement**

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46 All authors contributed to the study design and conception. AJu initiated the collection of the  
47  
48 prospective dataset. PK, AJā and NR were responsible for assessing statistical analyses. PK was a  
49  
50 major contributor in writing the manuscript. All authors provided comments on drafts of the  
51  
52 manuscript. All authors read and approved the final manuscript.  
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### 58 **Data sharing statement**

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5 No additional data available.  
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## 10 **Figure legends**

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14 Figure 1. Flow chart of the study participants.  
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19 Figure 2. Associations between primary tumor properties and survival in metastatic breast cancer.  
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21 In multivariate analysis, only ER expression and initial nodal status remained as significant factors.  
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## 31 **References**

32

33 1 Bray F, Ferlay J, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and  
34 mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.  
35

36 2 Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO International Consensus Guidelines for  
37 Advanced Breast Cancer (ABC 4) dagger. *Ann Oncol* 2018;29:1634-57.  
38

39 3 Cardoso F, Spence D, Mertz S, Corneliussen-James D, Sabelko K, Gralow J, et al. Global analysis  
40 of advanced/metastatic breast cancer: Decade report (2005-2015). *Breast* 2018;39:131-8.  
41  
42

43 4 Allemani C, Weir HK, Carreira H, et al. Global surveillance of cancer survival 1995-2009:  
44 analysis of individual data for 25,676,887 patients from 279 population-based registries in 67  
45 countries (CONCORD-2). *Lancet* 2015;385:977-1010.  
46  
47

48 5 Weigelt B, Peterse JL, van 't Veer, LJ. Breast cancer metastasis: markers and models. *Nat Rev*  
49 *Cancer* 2005;5:591-602.  
50

51 6 Early Breast Cancer Trialists' Collaborative Group, (EBCTCG), Peto R, Davies C, Godwin J,  
52 Gray R, Pan HC, et al. Comparisons between different polychemotherapy regimens for early breast  
53 cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials.  
54 *Lancet* 2012;379:432-44.  
55

56  
57 7 Chang J, Clark GM, Allred DC, et al. Survival of patients with metastatic breast carcinoma:  
58 importance of prognostic markers of the primary tumor. *Cancer* 2003;97:545-53.  
59  
60

- 1  
2  
3 8 Regierer AC, Wolters R, Ufen M, et al. An internally and externally validated prognostic score  
4 for metastatic breast cancer: analysis of 2269 patients. *Ann Oncol* 2014;25:633-8.  
5  
6 9 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast  
7 cancer. *Ann Oncol* 2008;19:2012-9.  
8  
9  
10 10 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-  
11 free interval is important. *Hum Pathol* 2017;70:70-6.  
12  
13 11 Stuart-Harris R, Shadbolt B, Palmqvist C, et al. The prognostic significance of single hormone  
14 receptor positive metastatic breast cancer: an analysis of three randomised phase III trials of  
15 aromatase inhibitors. *Breast* 2009;18:351-5.  
16  
17 12 Clark GM, Sledge GW, Jr, Osborne CK, et al. Survival from first recurrence: relative importance  
18 of prognostic factors in 1,015 breast cancer patients. *J Clin Oncol* 1987;5(1):55-61.  
19  
20 13 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-  
21 free interval is important. *Hum Pathol* 2017;70:70-6.  
22  
23 14 Lobbezoo DJA, van Kampen, RJW., Voogd AC, et al. Prognosis of metastatic breast cancer: are  
24 there differences between patients with de novo and recurrent metastatic breast cancer? *Br J Cancer*  
25 2015;112:1445-51.  
26  
27 15 Senkus E, Kyriakides S, Ohno S, et al. Primary breast cancer: ESMO Clinical Practice  
28 Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26 Suppl 5:8.  
29  
30 16 Karihtala P, Mäntyniemi A, Kang SW, et al. Peroxiredoxins in breast carcinoma. *Clin Cancer*  
31 *Res* 2003;9:3418-24.  
32  
33 17 Isola J, Tanner M, Forsyth A, et al. Interlaboratory comparison of HER-2 oncogene  
34 amplification as detected by chromogenic and fluorescence in situ hybridization. *Clin Cancer Res*  
35 2004;10:4793-8.  
36  
37 18 Yamamoto N, Watanabe T, Katsumata N, et al. Construction and validation of a practical  
38 prognostic index for patients with metastatic breast cancer. *J Clin Oncol* 1998;16:2401-8.  
39  
40 19 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast  
41 cancer patients: analysis of deceased patients. *Rad Oncol J* 2013;31:222-7.  
42  
43 20 Khanfir A, Lahiani F, Bouzguenda R, et al. Prognostic factors and survival in metastatic breast  
44 cancer: A single institution experience. *Rep Pract Oncol Radiother* 2013;18:127-32.  
45  
46 21 Hopkins AM, Rowland A, McKinnon RA, et al. Predictors of Long-Term Disease Control and  
47 Survival for HER2-Positive Advanced Breast Cancer Patients Treated With Pertuzumab,  
48 Trastuzumab, and Docetaxel. *Front Oncol* 2019;9:789.  
49  
50 22 Dawood S, Broglio K, Buzdar AU, et al. Prognosis of women with metastatic breast cancer by  
51 HER2 status and trastuzumab treatment: an institutional-based review. *J Clin Oncol* 2010;28:92-8.  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 23 King TA, Lyman JP, Gonen M, et al. Prognostic Impact of 21-Gene Recurrence Score in  
4 Patients With Stage IV Breast Cancer: TBCRC 013. *J Clin Oncol* 2016 Jul 10;34(20):2359-65.  
5  
6  
7 24 Prat A, Cheang MC, Galvan P, et al. Prognostic Value of Intrinsic Subtypes in Hormone  
8 Receptor-Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib.  
9 *JAMA Oncol* 2016;2:1287-94.  
10  
11 25 Papadaki C, Stoupis G, Tsalikis L, et al. Circulating miRNAs as a marker of metastatic disease  
12 and prognostic factor in metastatic breast cancer. *Oncotarget* 2019;10:966-81.  
13  
14  
15 26 Van Poznak C, Somerfield MR, Bast RC, et al. Use of Biomarkers to Guide Decisions on  
16 Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical  
17 Oncology Clinical Practice Guideline. *J Clin Oncol* 2015;33(24):2695-704.  
18  
19  
20 27 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast  
21 cancer. *Ann Oncol* 2008;19:2012-9.  
22  
23 28 Insa A, Lluch A, Prosper F, et al. Prognostic factors predicting survival from first recurrence in  
24 patients with metastatic breast cancer: analysis of 439 patients. *Breast Cancer Res Treat*  
25 1999;56(1):67-78.  
26  
27  
28 29 Tsuji W, Teramukai S, Ueno M, et al. Prognostic factors for survival after first recurrence in  
29 breast cancer: a retrospective analysis of 252 recurrent cases at a single institution. *Breast Cancer*  
30 2014;21:86-95.  
31  
32 30 Rack B, Janni W, Gerber B, et al. Patients with recurrent breast cancer: does the primary axillary  
33 lymph node status predict more aggressive tumor progression? *Breast Cancer Res Treat*  
34 2003;82(2):83-92.  
35  
36  
37 31 Puente J, Lopez-Tarruella S, Ruiz A, et al. Practical prognostic index for patients with metastatic  
38 recurrent breast cancer: retrospective analysis of 2,322 patients from the GEICAM Spanish El  
39 Alamo Register. *Breast Cancer Res Treat* 2010;122:591-600.  
40  
41  
42 32 Gibert-Ramos A, Lopez C, Bosch R, et al. Immune response profile of primary tumour, sentinel  
43 and non-sentinel axillary lymph nodes related to metastasis in breast cancer: an  
44 immunohistochemical point of view. *Histochem Cell Biol* 2019;152:177-93.  
45  
46  
47 33 Ullah I, Karthik G, Alkodsai A, et al. Evolutionary history of metastatic breast cancer reveals  
48 minimal seeding from axillary lymph nodes. *J Clin Invest* 2018;128:1355-70.  
49  
50  
51 34 Klein CA. Selection and adaptation during metastatic cancer progression. *Nature* 2013 Sep  
52 19;501:365-72.  
53  
54  
55 35 Engel J, Emeny RT, Hölzel D. Positive lymph nodes do not metastasize. *Cancer Metastasis Rev*  
56 2012;31:235-46.  
57  
58  
59 36 Barry P, Vatsiou A, Spiteri I, et al. The Spatiotemporal Evolution of Lymph Node Spread in  
60 Early Breast Cancer. *Clin Cancer Res* 2018;24:4763-70.



1  
2  
3 37 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast  
4 cancer patients: analysis of deceased patients. *Radiat Oncol J* 2013;31:222-7.  
5

6  
7 38 Woo JW, Chung YR, Ahn S, et al. Changes in Biomarker Status in Metastatic Breast Cancer and  
8 Their Prognostic Value. *J Breast Cancer* 2019;22:439-52.  
9

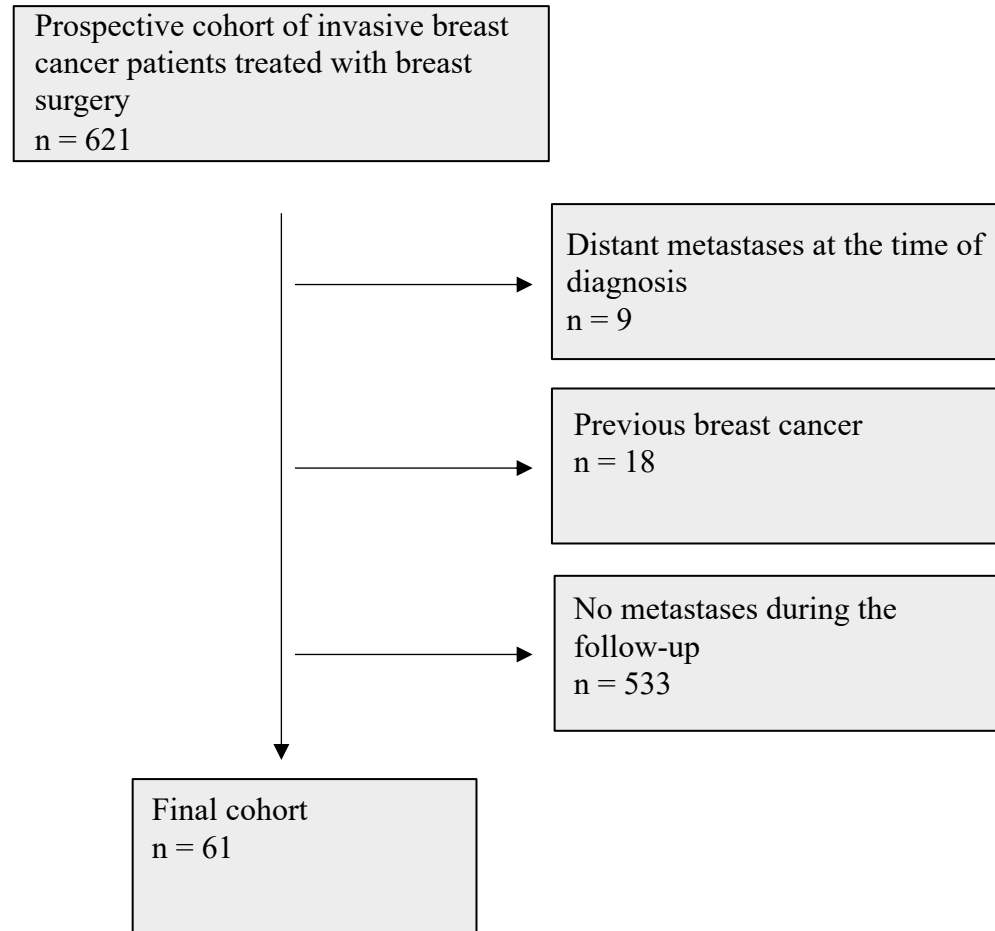
10  
11 39 Fietz T, Tesch H, Rauh J, et al. Palliative systemic therapy and overall survival of 1,395 patients  
12 with advanced breast cancer - Results from the prospective German TMK cohort study. *Breast*  
13 2017;34:122-30.

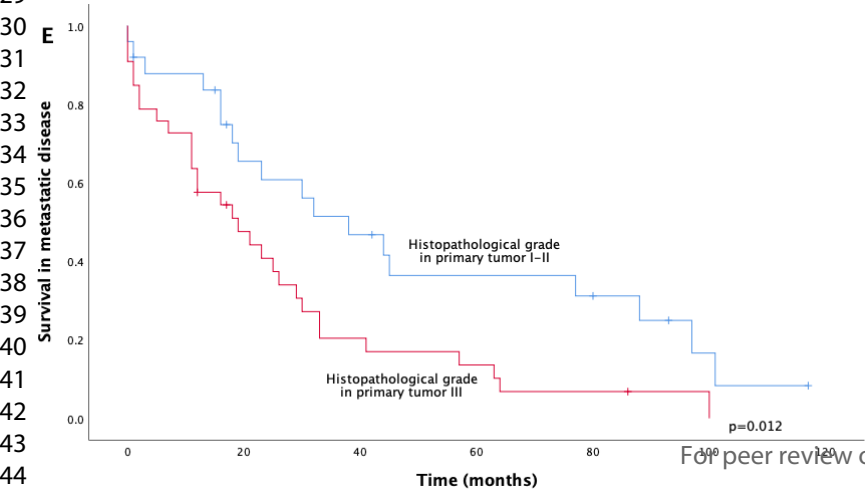
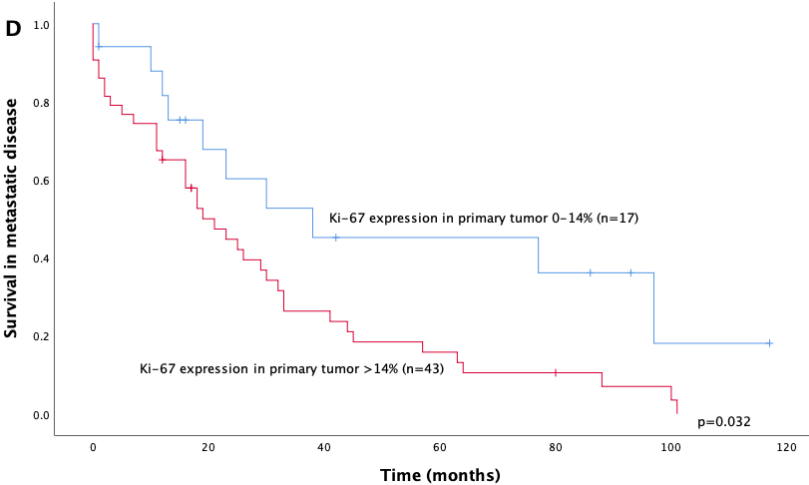
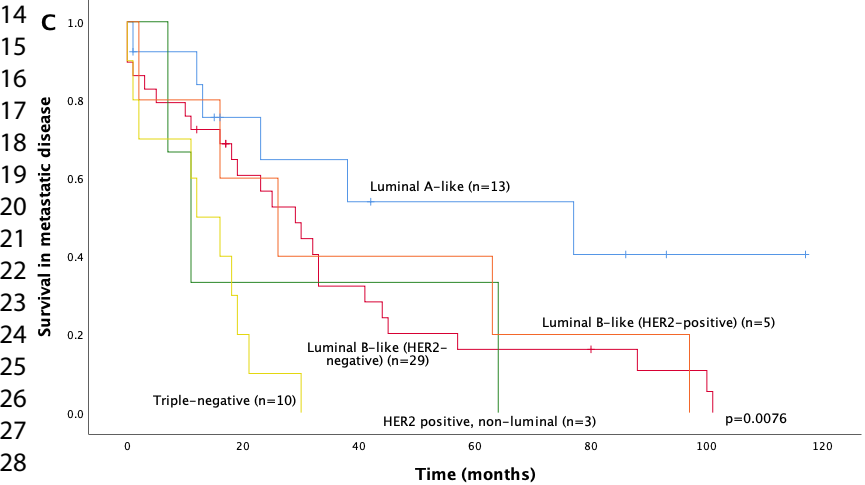
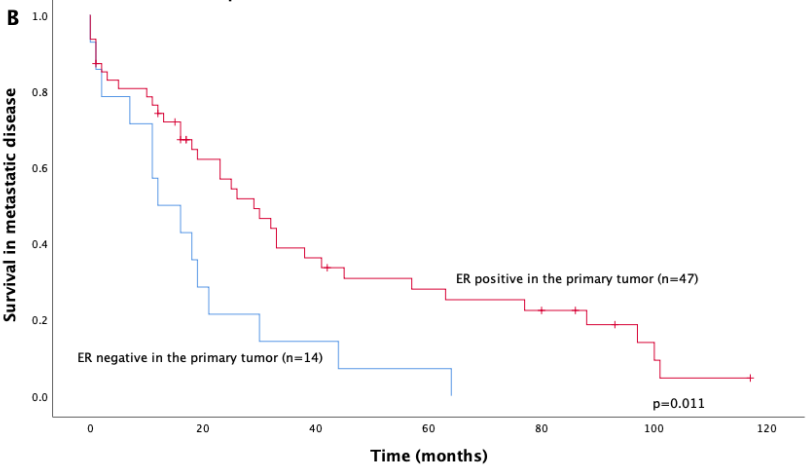
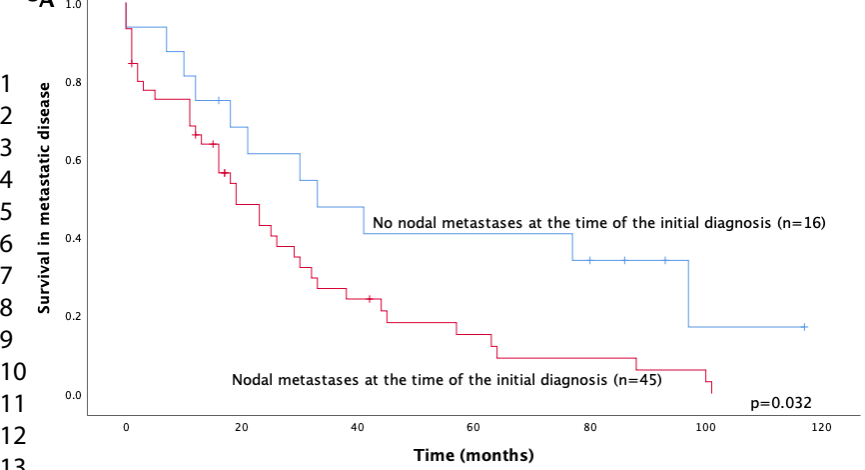
14  
15 40 Januskeviciene I, Petrikaite V. Heterogeneity of breast cancer: The importance of interaction  
16 between different tumor cell populations. *Life Sci* 2019;239:117009.  
17

18  
19 41 Breast Cancer [Internet].; 2019 [updated Sep 6,; cited Jan 12, 2019]. Available from:

20  
21 [https://www.nccn.org/professionals/physician\\_gls/pdf/breast.pdf](https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf).  
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## STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	a prospective single-center study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses		
<b>Methods</b>				
Study design	4	Present key elements of study design early in the paper	4	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3, 4	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4	
Bias	9	Describe any efforts to address potential sources of bias	5, 11	
Study size	10	Explain how the study size was arrived at	4	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4, 8, 9
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	5
		(b) Describe any methods used to examine subgroups and interactions	4, 5
		(c) Explain how missing data were addressed	5-7
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	3
		(c) Consider use of a flow diagram	As Figure
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-7
		(b) Indicate number of participants with missing data for each variable of interest	5-7
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	8
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(b) Report category boundaries when continuous variables were categorized	9
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	2, 3, 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-12
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Prognostic factors in metastatic breast cancer: a prospective single-center study

Journal:	<i>BMJ Open</i>
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<b>Primary Subject Heading</b>:	Oncology
Secondary Subject Heading:	Pathology
Keywords:	Breast tumours < ONCOLOGY, Adult oncology < ONCOLOGY, CHEMOTHERAPY

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3 Prognostic factors in metastatic breast cancer: a prospective single-center study  
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53 Keywords: estrogen receptor; metastatic breast cancer; metastasis; nodal status; prognosis  
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56 Word count: 2,549  
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## Abstract

**Objectives:** Although novel early breast cancer prognostic factors are being continuously discovered, only rare factors predicting survival in metastatic breast cancer have been validated. The prognostic role of early breast cancer prognostic factors in metastatic disease also remains mostly unclear.

**Design and setting:** Prospective study in a Finnish University Hospital.

**Participants and outcomes:** 594 women with early breast cancer were originally followed. Sixty-one of these patients developed distant metastases during the follow-up, and their primary breast cancer properties, such as tumor size, nodal status, estrogen (ER) and progesterone receptor expression, grade, proliferation rate, histopathological subtype and breast cancer subtype were analyzed as potential prognostic factors for metastatic disease.

**Results:** In multivariate analysis, the presence of lymph node metastases at the time of early breast cancer surgery (hazard ratio (HR), 2.17; 95% confidence interval (CI), 1.09-4.31;  $p = 0.027$ ) and ER status (negative versus positive, HR, 2.16; 95% CI, 1.14-4.10;  $p = 0.018$ ) were significant predictors of survival in metastatic disease.

**Conclusions:** These results confirm ER status as a primary prognostic factor in metastatic breast cancer. Furthermore, it also suggests that the presence of initial lymph node metastases could serve as a prognostic factor in recurrent breast cancer.

## Article summary

### Strengths and limitations of this study

- This was a study of contemporary, prospective breast cancer cohort in a University Hospital with a relatively long follow-up.
- The material did not include patients with *de novo* metastatic breast cancer.

- The number of patients with metastatic breast cancer should have been larger to make more detailed subgroup analyses, regarding e.g. biological subgroups possible.

## Introduction

Breast cancer is by far the most common and deadliest cancer affecting women worldwide [1]. In contrast to considerably prolonged early breast cancer prognosis during the last decade, which is mainly due to the optimization of adjuvant therapies, the prognosis of patients with metastatic breast cancer has hardly been prolonged, and the current median of overall survival is approximately 36 months [2-5].

The presence of nodal involvement is the strongest predictor of outcomes for early breast cancer [6]. The clinical behavior of metastatic breast cancer still varies greatly, and it is difficult to predict. The best validated prognostic factors in metastatic breast cancer include clinical factors, such as long relapse-free intervals, the absence of brain metastases or visceral metastases and the presence of estrogen receptor (ER), which also serves as an essential predictive factor in metastatic settings [7-12]. *De novo* metastatic breast cancers also have better prognosis than recurrent breast cancer [13, 14]. The possibility of using other characteristics of primary breast cancer, such as primary tumor size and axillary lymph node status as prognostic factors in metastatic breast cancer is still being discussed; however, this approach has seldom been studied in modern prospective cohorts.

Using a large prospective breast cancer cohort treated with modern treatment modalities, we aimed to determine whether primary breast cancer prognostic factors, such as tumor size, nodal status, ER and progesterone receptor (PR) expression, differentiation, proliferation rate or breast cancer subtype could also predict outcomes in recurrent metastatic breast cancer.

## Materials and methods

The original patient material was from a prospective dataset collected in Oulu University Hospital from 2003–2013. The dataset consisted of 594 patients with early invasive breast cancer diagnosed and treated in Oulu University Hospital, Finland. Surgery to the primary tumor was carried out according to the guidelines of Finnish Breast Cancer Group. The dataset did not include information of the possible neoadjuvant chemotherapy, which was nevertheless very rarely administered during the study period. Patients with previous breast cancer or distant metastases at the time of diagnosis were excluded from the cohort (Figure 1). During the follow-up, 61 women displayed distant metastases, and the outcomes of these patients were reported in this study.

Tumors were classed into five intrinsic subtypes according to European Society for Medical Oncology Clinical Practice Guidelines on Breast Cancer [15]. Luminal A-like carcinomas expressed ER and PR, showed Ki-67 expression in < 15% of the cells, and did not overexpress HER2. Luminal B-like (HER2-negative) carcinomas were ER-positive and HER2-negative. In addition, they showed either Ki-67 expression in  $\geq 15\%$  of cells, or they were PR-negative. Luminal B-like (HER2-positive) tumors expressed ER and overexpressed HER2. Triple-negative breast carcinomas (TNBC) were defined as tumors with no ER, PR and HER2 expression. HER2-positive (non-luminal) cases overexpressed HER2 without ER or PR positivity. The distribution between subtypes in the cohort is described in detail in Table 1.

The histopathology was evaluated according to current WHO classification and stage was assessed using TNM classification. The expressions of ER, PR and Ki-67 were studied using

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3 immunohistochemistry as previously described [16]. HER2 expression was studied using  
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5 immunohistochemistry and chromogenic in situ hybridization (CISH) to confirm positive results. A  
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7 positive result of six or more gene copies in CISH was considered HER2-positive [17] .  
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## 10 11 12 Ethical considerations

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17 The patients provided their written informed consent to participate the study. The study was  
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19 approved by the Local Ethics Committee of the Ostrobothnia Hospital District (114/2011) and the  
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21 National Supervisory Authority for Welfare and Health (D9580/05.01.00.06/2010). All studies were  
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23 conducted in accordance with the principles of the Declaration of Helsinki and the guidelines for  
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25 good clinical practice.  
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## 30 31 Statistical analyses

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35 Statistical analysis was performed using IBM SPSS Statistics software, v. 25.0.0.0 for Mac (IBM  
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37 Corporation, Armonk, NY, USA). Survival was analyzed with Kaplan-Meier curves and the log-  
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39 rank tests. Correction was not made for multiple comparisons. Survival in metastatic disease was  
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41 calculated from the date when metastasis was first observed in imaging to the time of death.  
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45 Multivariate analysis was conducted using Cox multivariate regression analysis. P-values less than  
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47 0.05 were considered significant.  
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52 Table 1. Primary tumor characteristics.

	N (%)
<b>Tumor size</b>	
T1	20 (32.8%)

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T2	33 (54.1%)
T3	7 (11.5%)
T4	1 (1.6%)
<b>Nodal status</b>	
N0	16 (26.2%)
N1	22 (36.1%)
N2	15 (24.6%)
N3	8 (13.1%)
<b>Histopathology</b>	
Ductal	47 (77.0%)
Lobular	11 (18.0%)
Other	3 (4.9%)
<b>Histopathological grade</b>	
Grade 1	0 (0%)
Grade 2	25 (41.0%)
Grade 3	34 (55.7%)
Unknown	2 (3.3%)
<b>ER expression</b>	
Negative (0 %)	14 (23.0%)
Weak (1-9 %)	2 (3.3%)
Moderate (10-59 %)	6 (9.8%)
High (>59 %)	39 (63.9%)
<b>PR expression</b>	
Negative (0 %)	22 (36.1%)

Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
<b>HER2 status</b>	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
<b>Ki-67 expression</b>	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
<b>Focality</b>	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
<b>Subtype</b>	
Luminal A-like	13 (21.3%)
Luminal B-like (HER2-negative)	29 (47.5%)
Luminal B-like (HER2-positive)	5 (8.2%)
HER2-positive, non-luminal	3 (4.9%)
Triple-negative	10 (16.4%)
Unknown	1 (1.6%)
<b>The first site of the distant metastasis</b>	
Bone only	17 (27.9%)
Lung only	9 (14.8%)



Liver only	5 (8.2%)
Other	6 (9.6%)
Multiple sites	24 (39.3%)

## Patient and public involvement

Patients or public were not involved in the design, conduct, reporting or dissemination of this study.

## Results

Sixty-one patients of the originally 594 women ultimately developed distant metastases during the follow-up. Of these, fifty patients died of breast cancer during the follow-up. The median disease-free interval was 39.0 months in the patients who had distant metastases. The median follow-up time starting from the early breast cancer diagnosis was 72.0 months in patients who later developed metastases.

The median follow-up of the patients during their metastatic breast cancer was 18.0 months (mean 30.2 months). The Kaplan-Meier estimate for median survival of the patients with metastatic breast cancer was 77.0 months in those with luminal A-like breast cancers, 29.0 months in those with luminal B-like (HER2-negative) disease and 11.0, 26.0 and 12.0 months in those with HER2-positive, non-luminal, luminal B-like (HER2-positive) and TNBC subtype, respectively.

Patients with metastatic local lymph nodes at the time of definitive surgery displayed poorer survival outcomes for metastatic disease ( $p = 0.031$ ) (Figure 2). The Kaplan-Meier estimate for median survival in metastatic disease in lymph node-negative patients was 33.0 months, and in

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3 lymph node-positive patients, it was 19.0 months. Only N0 versus N1-3 classification was  
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5 significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes ( $p =$   
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7 0.78) were detected.  
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12 Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary  
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14 tumor ( $p = 0.011$ ), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors ( $p$   
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16  $= 0.032$ ) and grade I–II (versus grade III) primary tumors ( $p = 0.012$ ) were associated with better  
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18 survival in metastatic disease in univariate analysis. Breast cancer subtype (determined from the  
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20 initial surgical samples) also predicted survival with metastatic breast cancer ( $p = 0.0078$ ) (Figure  
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22 2C). Also, the patients with luminal A-like breast cancer had significantly prolonged survival, when  
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24 compared to all other subtypes ( $p = 0.017$ ). Primary tumor size, PR or HER2 expression, the site of  
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26 the first metastasis in bone versus elsewhere, disease-free interval ( $\leq 24$  months versus  $> 24$   
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28 months) or age at disease onset were not associated with metastatic disease survival.  
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35 When assessed separately by different biological subtypes, initial lymph node metastases predicted  
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37 worse prognosis only in the patients with the luminal A subtype in univariate analysis ( $p = 0.019$ ),  
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39 but the small sample size of each subgroup limited the reliability of this analysis (data not shown).  
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45 In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis  
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47 predicted poorer survival overall (HR, 2.17; 95% CI, 1.09-4.31;  $p = 0.027$ ) when tumor size (T1  
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49 versus T2-4) (HR, 1.33; 95% CI, 0.71-2.47;  $p = 0.37$ ) and ER status (negative versus positive) (HR  
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51 2.16; 95% CI 1.14-4.10;  $p = 0.018$ ) were included in the analysis. The proportional hazards  
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53 assumption was met in the analysis. Breast cancer subtype, Ki-67 expression or grade did not  
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55 remain significant prognostic factors after multivariate analysis.  
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## Discussion

As the main observation, we report in this prospectively collected and contemporary cohort from a Finnish University Hospital that the presence of local lymph node metastasis at the time of early breast cancer surgery predicted short survival in subsequent metastatic breast cancer. Our results also supported previous results of ER negativity in primary breast cancer as an adverse prognostic factor for disease recurrence.

The most established prognostic factors of better outcome in metastatic breast cancer include ER positivity, long disease-free interval (usually defined as at least 2 years), low number of metastatic sites and bone-only localization of metastases [7-14, 18-20]. HER2 appears to no longer represent a prognostic factor in the era of targeted treatments, and prognostic factors also slightly differ between HER2-positive and HER2-negative patients [21, 22]. Emerging metastatic breast cancer prognostic factors include circulating tumor cells, gene expression panels, circulating tumor markers and miRNAs; however, they have not yet been sufficiently validated [23-26]. Whereas ER status, a lengthy disease-free interval and metastatic load are established and obvious prognostic factors for metastatic breast cancer, the presence of lymph node metastases at the time of initial diagnosis has not been widely studied in metastatic breast cancer, although it is the strongest prognostic factor in early breast cancer. In the current study, we concentrated solely on primary breast cancer characteristics, and we did not assess other characteristics, such as disease-free interval, metastasis load or metastasis location as prognostic factors.

Some previous studies with mostly retrospective cohort settings and outdated treatment modalities have reported the initial nodal status as a prognostic factor in metastatic breast cancer, whereas others have not found such an association [7, 27-29]. In the pioneer work of Clark et al., nodal

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3 involvement at time of initial diagnosis was associated with shorter survival [12]. Another  
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5 retrospective single-institute study also concluded that lymph node involvement at primary  
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7 diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients  
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9 were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish  
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11 retrospective registry study suggested that the initial lymph node status should be part of the  
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13 prognostic index in recurrent metastatic breast cancer [31]. In addition to considerable change in the  
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15 oncological treatments of breast cancer during the last decades, also surgical techniques, especially  
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17 axillary procedures have developed considerably. The current results from the prospective data with  
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19 modern treatments thus support and confirm earlier results.  
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26 In our study, any metastasis found in local lymph nodes at the time of definitive surgery was  
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28 associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median  
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30 survival was prolonged from 19–33 months in patients without lymph node metastases at the time  
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32 of initial diagnosis. Although lymph node metastases in general is associated with other factors of  
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34 poor prognosis, our study suggested that this result was independent of tumor size and ER  
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36 expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it  
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38 can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent  
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40 paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions  
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42 suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for  
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44 predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per*  
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46 *se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually  
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48 metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary  
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50 lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these  
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52 issues continues [36].  
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3 It has to be emphasized that all our patients had recurrent breast cancer, and our material did not  
4 include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the  
5 material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast  
6 cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless,  
7 the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer,  
8 and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9,  
9 37]. As an other limitation, we were unable to address the results separately in subgroups, for  
10 example according to biological subtypes, due to relatively low number of patients with metastatic  
11 breast cancer. On the other hand, our study was based on a prospective cohort from a university  
12 clinic, and the patients were treated with up-to-date surgical and oncological treatment modalities.  
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28 Our results confirmed that ER negativity in primary tumor was associated with short survival for  
29 metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but  
30 also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER  
31 negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12,  
32 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER  
33 status is also a predictor of poor prognosis [38]. Most previous studies have divided metastatic  
34 breast cancers only to three subgroups: ER/PR-positive, HER2-positive and TNBC. We used the  
35 widely recognized ESMO guidelines for subtyping our cases. Although the number of patients in  
36 each subgroup were rather limited, the patients with slowly proliferating, ER-responsive luminal A-  
37 like breast cancers still had significantly prolonged survival in metastatic breast cancer compared to  
38 other subtypes. TNBC has the worst outcome of all subtypes in metastatic breast cancer, a finding  
39 which was mirrored in our study [39].  
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3 Predicting the course of metastatic breast cancer is of primary importance in clinical practice;  
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5 however, its status as a highly heterogenous disease at both the inpatient and outpatient levels  
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7 makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer  
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9 guidelines recommend starting treatment with chemotherapy or even with a combination  
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11 chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing  
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13 ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic  
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15 breast cancer, such as initial nodal status, could be confirmed, these patients should receive more  
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17 aggressive first-line metastatic breast cancer therapy.  
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24 In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse  
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26 prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial  
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28 lymph node status may be a prognostic factor for metastatic disease course. Future studies should  
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30 also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute  
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32 number of metastatic lymph nodes, which were not addressed in our material. More research is also  
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34 clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or  
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36 whether they are purely an indicator of aggressive disease.  
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### 42 **Contributorship statement**

43  
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45  
46 All authors contributed to the study design and conception. AJu initiated the collection of the  
47  
48 prospective dataset. PK, AJä and NR were responsible for assessing statistical analyses. PK was a  
49  
50 major contributor in writing the manuscript. All authors provided comments on drafts of the  
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52 manuscript. All authors read and approved the final manuscript.  
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### 58 **Competing interests**

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5 The authors report no competing interests.  
6  
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11  
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14 There are no funders to report for this submission.  
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## 18 **Data sharing statement**

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22 Data are available upon reasonable request.  
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## 28 **Figure legends**

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33 Figure 1. Flow chart of the study participants.  
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37 Figure 2. Associations between primary tumor properties and survival in metastatic breast cancer.  
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40 In multivariate analysis, only ER expression and initial nodal status remained as significant factors.  
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## 50 **References**

51 1 Bray F, Ferlay J, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and  
52 mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.  
53

54 2 Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO International Consensus Guidelines for  
55 Advanced Breast Cancer (ABC 4)dagger. *Ann Oncol* 2018;29:1634-57.  
56  
57

58 3 Cardoso F, Spence D, Mertz S, Corneliussen-James D, Sabelko K, Gralow J, et al. Global analysis  
59 of advanced/metastatic breast cancer: Decade report (2005-2015). *Breast* 2018;39:131-8.  
60

- 1  
2  
3 4 Allemani C, Weir HK, Carreira H, et al. Global surveillance of cancer survival 1995-2009:  
4 analysis of individual data for 25,676,887 patients from 279 population-based registries in 67  
5 countries (CONCORD-2). *Lancet* 2015;385:977-1010.  
6
- 7  
8 5 Weigelt B, Peterse JL, van 't Veer, LJ. Breast cancer metastasis: markers and models. *Nat Rev*  
9 *Cancer* 2005;5:591-602.  
10
- 11 6 Early Breast Cancer Trialists' Collaborative Group, (EBCTCG), Peto R, Davies C, Godwin J,  
12 Gray R, Pan HC, et al. Comparisons between different polychemotherapy regimens for early breast  
13 cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials.  
14 *Lancet* 2012;379:432-44.  
15
- 16  
17 7 Chang J, Clark GM, Allred DC, et al. Survival of patients with metastatic breast carcinoma:  
18 importance of prognostic markers of the primary tumor. *Cancer* 2003;97:545-53.  
19
- 20  
21 8 Regierer AC, Wolters R, Ufen M, et al. An internally and externally validated prognostic score  
22 for metastatic breast cancer: analysis of 2269 patients. *Ann Oncol* 2014;25:633-8.  
23
- 24 9 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast  
25 cancer. *Ann Oncol* 2008;19:2012-9.  
26
- 27  
28 10 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-  
29 free interval is important. *Hum Pathol* 2017;70:70-6.  
30
- 31 11 Stuart-Harris R, Shadbolt B, Palmqvist C, et al. The prognostic significance of single hormone  
32 receptor positive metastatic breast cancer: an analysis of three randomised phase III trials of  
33 aromatase inhibitors. *Breast* 2009;18:351-5.  
34
- 35  
36 12 Clark GM, Sledge GW, Jr, Osborne CK, et al. Survival from first recurrence: relative importance  
37 of prognostic factors in 1,015 breast cancer patients. *J Clin Oncol* 1987;5(1):55-61.  
38
- 39 13 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-  
40 free interval is important. *Hum Pathol* 2017;70:70-6.  
41
- 42  
43 14 Lobbezoo DJA, van Kampen, RJW., Voogd AC, et al. Prognosis of metastatic breast cancer: are  
44 there differences between patients with de novo and recurrent metastatic breast cancer? *Br J Cancer*  
45 2015;112:1445-51.  
46
- 47 15 Senkus E, Kyriakides S, Ohno S, et al. Primary breast cancer: ESMO Clinical Practice  
48 Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26 Suppl 5:8.  
49
- 50  
51 16 Karihtala P, Mäntyniemi A, Kang SW, et al. Peroxiredoxins in breast carcinoma. *Clin Cancer*  
52 *Res* 2003;9:3418-24.  
53
- 54 17 Isola J, Tanner M, Forsyth A, et al. Interlaboratory comparison of HER-2 oncogene  
55 amplification as detected by chromogenic and fluorescence in situ hybridization. *Clin Cancer Res*  
56 2004;10:4793-8.  
57
- 58  
59 18 Yamamoto N, Watanabe T, Katsumata N, et al. Construction and validation of a practical  
60 prognostic index for patients with metastatic breast cancer. *J Clin Oncol* 1998;16:2401-8.



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60

19 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast cancer patients: analysis of deceased patients. *Rad Oncol J* 2013;31:222-7.

20 Khanfir A, Lahiani F, Bouzguenda R, et al. Prognostic factors and survival in metastatic breast cancer: A single institution experience. *Rep Pract Oncol Radiother* 2013;18:127-32.

21 Hopkins AM, Rowland A, McKinnon RA, et al. Predictors of Long-Term Disease Control and Survival for HER2-Positive Advanced Breast Cancer Patients Treated With Pertuzumab, Trastuzumab, and Docetaxel. *Front Oncol* 2019;9:789.

22 Dawood S, Broglio K, Buzdar AU, et al. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. *J Clin Oncol* 2010;28:92-8.

23 King TA, Lyman JP, Gonen M, et al. Prognostic Impact of 21-Gene Recurrence Score in Patients With Stage IV Breast Cancer: TBCRC 013. *J Clin Oncol* 2016 Jul 10;34(20):2359-65.

24 Prat A, Cheang MC, Galvan P, et al. Prognostic Value of Intrinsic Subtypes in Hormone Receptor-Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib. *JAMA Oncol* 2016;2:1287-94.

25 Papadaki C, Stoupis G, Tsalikis L, et al. Circulating miRNAs as a marker of metastatic disease and prognostic factor in metastatic breast cancer. *Oncotarget* 2019;10:966-81.

26 Van Poznak C, Somerfield MR, Bast RC, et al. Use of Biomarkers to Guide Decisions on Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2015;33(24):2695-704.

27 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast cancer. *Ann Oncol* 2008;19:2012-9.

28 Insa A, Lluch A, Prosper F, et al. Prognostic factors predicting survival from first recurrence in patients with metastatic breast cancer: analysis of 439 patients. *Breast Cancer Res Treat* 1999;56(1):67-78.

29 Tsuji W, Teramukai S, Ueno M, et al. Prognostic factors for survival after first recurrence in breast cancer: a retrospective analysis of 252 recurrent cases at a single institution. *Breast Cancer* 2014;21:86-95.

30 Rack B, Janni W, Gerber B, et al. Patients with recurrent breast cancer: does the primary axillary lymph node status predict more aggressive tumor progression? *Breast Cancer Res Treat* 2003;82(2):83-92.

31 Puente J, Lopez-Tarruella S, Ruiz A, et al. Practical prognostic index for patients with metastatic recurrent breast cancer: retrospective analysis of 2,322 patients from the GEICAM Spanish El Alamo Register. *Breast Cancer Res Treat* 2010;122:591-600.

32 Gibert-Ramos A, Lopez C, Bosch R, et al. Immune response profile of primary tumour, sentinel and non-sentinel axillary lymph nodes related to metastasis in breast cancer: an immunohistochemical point of view. *Histochem Cell Biol* 2019;152:177-93.

1  
2  
3 33 Ullah I, Karthik G, Alkodsai A, et al. Evolutionary history of metastatic breast cancer reveals  
4 minimal seeding from axillary lymph nodes. *J Clin Invest* 2018;128:1355-70.  
5

6  
7 34 Klein CA. Selection and adaptation during metastatic cancer progression. *Nature* 2013 Sep  
8 19;501:365-72.  
9

10  
11 35 Engel J, Emeny RT, Hölzel D. Positive lymph nodes do not metastasize. *Cancer Metastasis Rev*  
12 2012;31:235-46.

13  
14 36 Barry P, Vatsiou A, Spiteri I, et al. The Spatiotemporal Evolution of Lymph Node Spread in  
15 Early Breast Cancer. *Clin Cancer Res* 2018;24:4763-70.  
16

17  
18 37 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast  
19 cancer patients: analysis of deceased patients. *Radiat Oncol J* 2013;31:222-7.

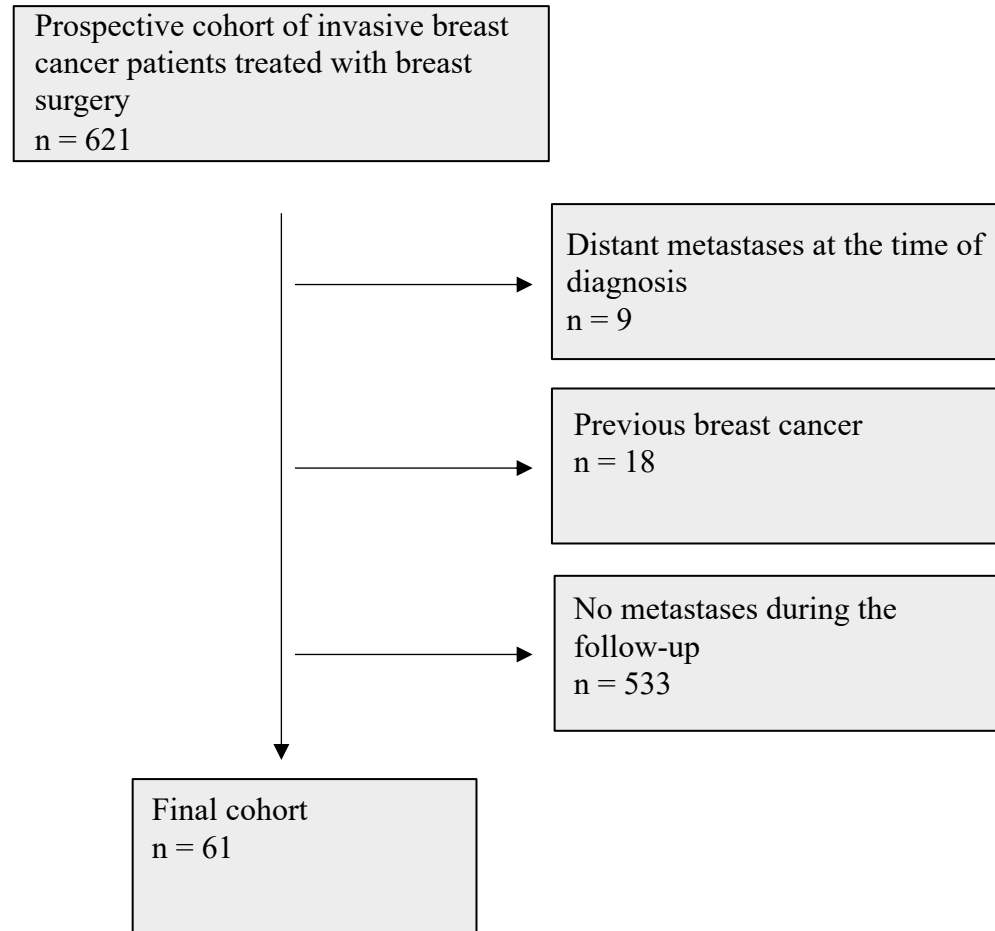
20  
21 38 Woo JW, Chung YR, Ahn S, et al. Changes in Biomarker Status in Metastatic Breast Cancer and  
22 Their Prognostic Value. *J Breast Cancer* 2019;22:439-52.  
23

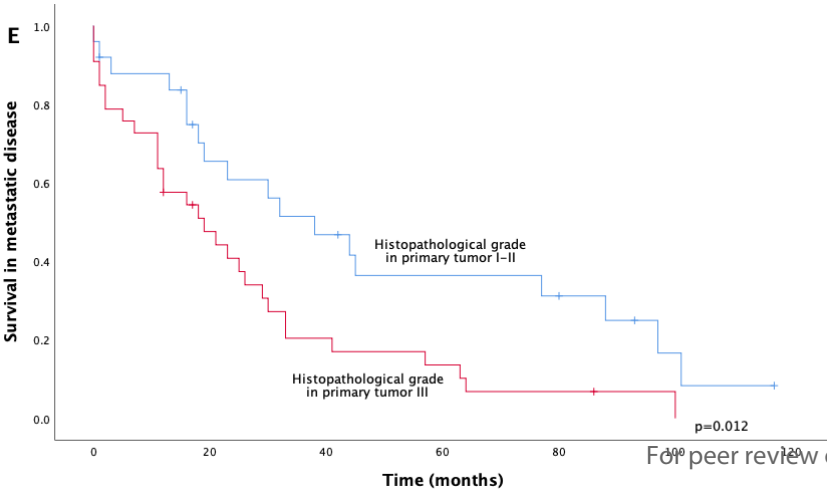
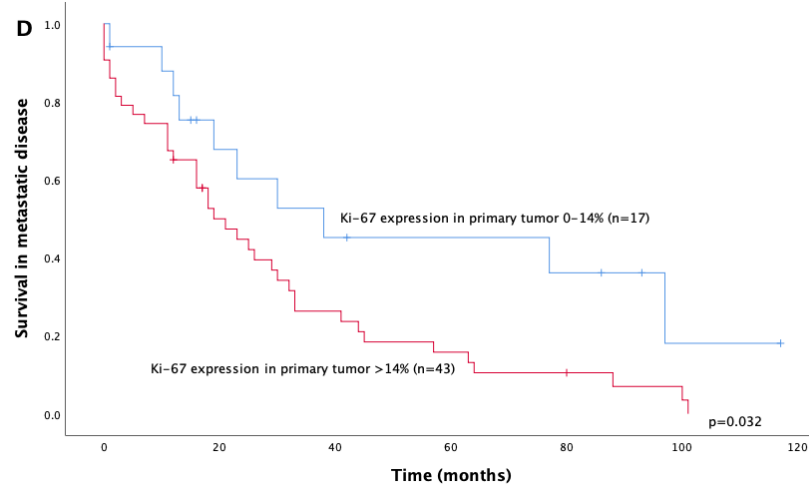
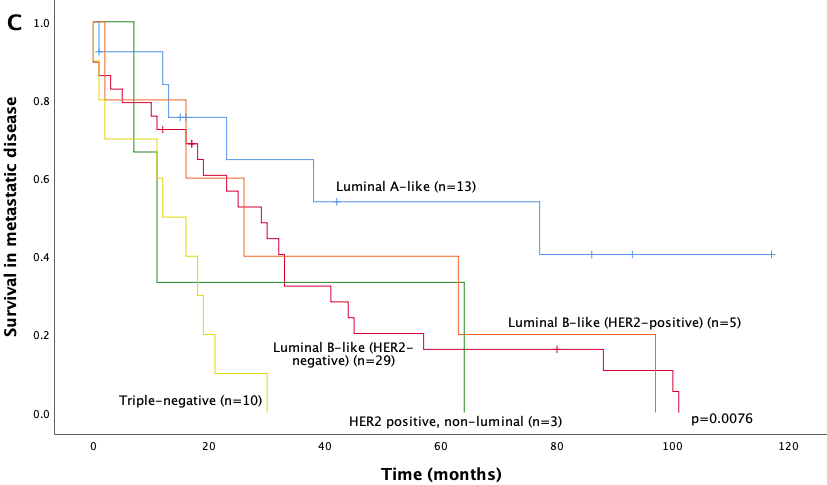
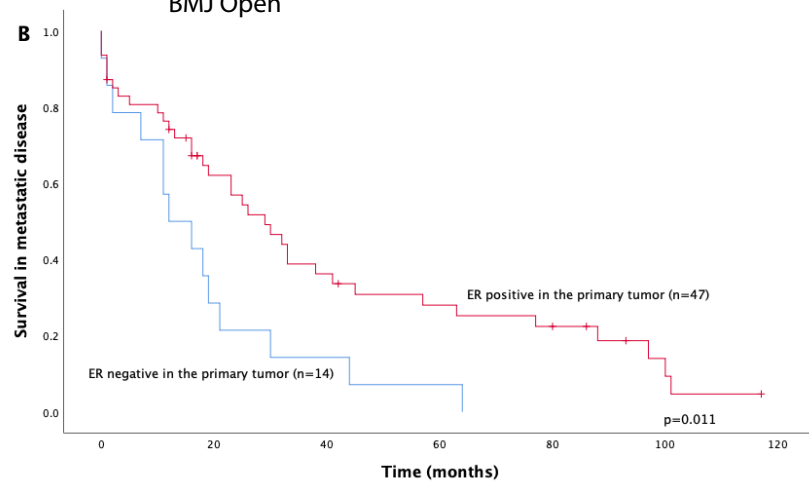
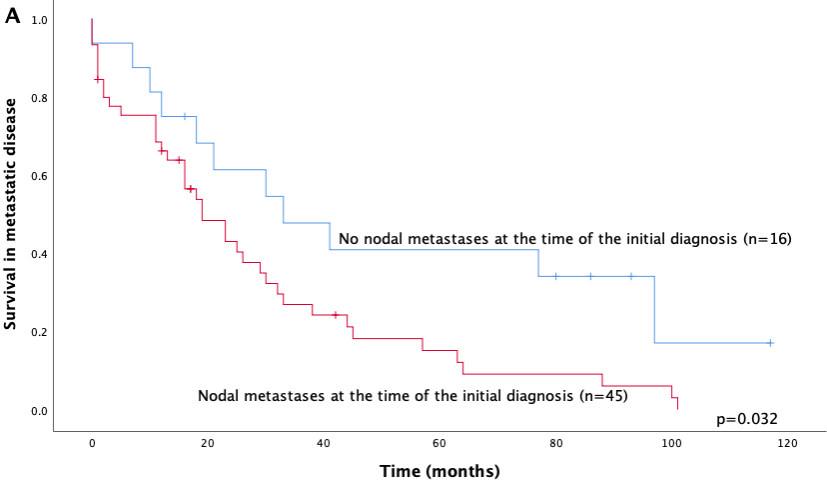
24  
25 39 Fietz T, Tesch H, Rauh J, et al. Palliative systemic therapy and overall survival of 1,395 patients  
26 with advanced breast cancer - Results from the prospective German TMK cohort study. *Breast*  
27 2017;34:122-30.  
28

29  
30 40 Januskeviciene I, Petrikaite V. Heterogeneity of breast cancer: The importance of interaction  
31 between different tumor cell populations. *Life Sci* 2019;239:117009.

32  
33 41 Breast Cancer [Internet].; 2019 [updated Sep 6,; cited Jan 12, 2019]. Available from:

34  
35 [https://www.nccn.org/professionals/physician\\_gls/pdf/breast.pdf](https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf).  
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## STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	a prospective single-center study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses		
<b>Methods</b>				
Study design	4	Present key elements of study design early in the paper	4	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3, 4	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4	
Bias	9	Describe any efforts to address potential sources of bias	5, 11	
Study size	10	Explain how the study size was arrived at	4	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4, 8, 9
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	5
		(b) Describe any methods used to examine subgroups and interactions	4, 5
		(c) Explain how missing data were addressed	5-7
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	3
		(c) Consider use of a flow diagram	As Figure
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-7
		(b) Indicate number of participants with missing data for each variable of interest	5-7
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	8
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(b) Report category boundaries when continuous variables were categorized	9
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	2, 3, 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-12
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Prognostic factors in metastatic breast cancer: a prospective single-center cohort study in a Finnish University Hospital

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3 Prognostic factors in metastatic breast cancer: a prospective single-center cohort study in a Finnish  
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56 Keywords: estrogen receptor; metastatic breast cancer; metastasis; nodal status; prognosis  
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58 Word count: 2,274  
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## Abstract

**Objectives:** Although novel early breast cancer prognostic factors are being continuously discovered, only rare factors predicting survival in metastatic breast cancer have been validated. The prognostic role of early breast cancer prognostic factors in metastatic disease also remains mostly unclear.

**Design and setting:** Prospective cohort study in a Finnish University Hospital.

**Participants and outcomes:** 594 women with early breast cancer were originally followed. Sixty-one of these patients developed distant metastases during the follow-up, and their primary breast cancer properties, such as tumor size, nodal status, estrogen (ER) and progesterone receptor expression, grade, proliferation rate, histopathological subtype and breast cancer subtype were analyzed as potential prognostic factors for metastatic disease.

**Results:** In multivariate analysis, the presence of lymph node metastases at the time of early breast cancer surgery (hazard ratio (HR), 2.17; 95% confidence interval (CI), 1.09-4.31;  $p = 0.027$ ) and ER status (negative versus positive, HR, 2.16; 95% CI, 1.14-4.10;  $p = 0.018$ ) were significant predictors of survival in metastatic disease.

**Conclusions:** These results confirm ER status as a primary prognostic factor in metastatic breast cancer. Furthermore, it also suggests that the presence of initial lymph node metastases could serve as a prognostic factor in recurrent breast cancer.

## Article summary

### Strengths and limitations of this study

- This study included contemporary, prospective breast cancer cohort in a University Hospital with a relatively long follow-up.

- The material did not include patients with *de novo* metastatic breast cancer.
- If the number of patients with metastatic breast cancer would have been larger, more detailed subgroup analyses, regarding e.g. biological subgroups possible, would have been possible.

## Introduction

Breast cancer is by far the most common and deadliest cancer affecting women worldwide [1]. In contrast to considerably prolonged early breast cancer prognosis during the last decade, which is mainly due to the optimization of adjuvant therapies, the prognosis of patients with metastatic breast cancer has hardly been prolonged, and the current median of overall survival is approximately 36 months [2-5].

The presence of nodal involvement is the strongest predictor of outcomes for early breast cancer [6]. The clinical behavior of metastatic breast cancer still varies greatly, and it is difficult to predict. The best validated prognostic factors in metastatic breast cancer include clinical factors, such as long relapse-free intervals, the absence of brain metastases or visceral metastases and the presence of estrogen receptor (ER), which also serves as an essential predictive factor in metastatic settings [7-12]. *De novo* metastatic breast cancers also have better prognosis than recurrent breast cancer [13, 14]. The possibility of using other characteristics of primary breast cancer, such as primary tumor size and axillary lymph node status as prognostic factors in metastatic breast cancer is still being discussed; however, this approach has seldom been studied in modern prospective cohorts.

Using a large prospective breast cancer cohort treated with modern treatment modalities, we aimed to determine whether primary breast cancer prognostic factors, such as tumor size, nodal status, ER

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3 and progesterone receptor (PR) expression, differentiation, proliferation rate or breast cancer  
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5 subtype could also predict outcomes in recurrent metastatic breast cancer.  
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## 10 **Materials and methods**

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15 The original patient material was from a prospective dataset collected in Oulu University Hospital  
16 from 2003–2013. The dataset consisted of 594 patients with early invasive breast cancer diagnosed  
17 and treated in Oulu University Hospital, Finland. Surgery to the primary tumor was carried out  
18 according to the guidelines of the Finnish Breast Cancer Group. The dataset did not include  
19 information of the possible neoadjuvant chemotherapy, which was nevertheless very rarely  
20 administered during the study period. Patients with previous breast cancer or distant metastases at  
21 the time of diagnosis were excluded from the cohort (Figure 1). During the follow-up, 61 women  
22 displayed distant metastases, and the outcomes of these patients were reported in this study.  
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38 Tumors were classed into five intrinsic subtypes according to the European Society for Medical  
39 Oncology Clinical Practice Guidelines on Breast Cancer [15]. Luminal A-like carcinomas  
40 expressed ER and PR, showed Ki-67 expression in  $< 15\%$  of the cells, and did not overexpress  
41 HER2. Luminal B-like (HER2-negative) carcinomas were ER-positive and HER2-negative. In  
42 addition, they showed either Ki-67 expression in  $\geq 15\%$  of cells, or they were PR-negative. Luminal  
43 B-like (HER2-positive) tumors expressed ER and overexpressed HER2. Triple-negative breast  
44 carcinomas (TNBC) were defined as tumors with no ER, PR and HER2 expression. HER2-positive  
45 (non-luminal) cases overexpressed HER2 without ER or PR positivity. The distribution between  
46 subtypes in the cohort is described in detail in Table 1.  
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3 The histopathology was evaluated according to current WHO classification and stage was assessed  
4 using TNM classification. The expressions of ER, PR and Ki-67 were studied using  
5 immunohistochemistry as previously described [16]. HER2 expression was studied using  
6 immunohistochemistry and chromogenic in situ hybridization (CISH) to confirm positive results. A  
7 positive result of six or more gene copies in CISH was considered HER2-positive [17] .  
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### 17 Ethical considerations

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21 The patients provided their written informed consent to participate the study. The study was  
22 approved by the Local Ethics Committee of the Ostrobothnia Hospital District (114/2011) and the  
23 National Supervisory Authority for Welfare and Health (D9580/05.01.00.06/2010). All studies were  
24 conducted in accordance with the principles of the Declaration of Helsinki and the guidelines for  
25 good clinical practice.  
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### 35 Statistical analyses

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39 Statistical analysis was performed using IBM SPSS Statistics software, v. 25.0.0.0 for Mac (IBM  
40 Corporation, Armonk, NY, USA). Survival was analyzed with Kaplan-Meier curves and the log-  
41 rank tests. Correction for multiple comparisons was not made. Survival in metastatic disease was  
42 calculated from the date when metastasis was first observed in imaging to the time of death.  
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49 Multivariate analysis was conducted using Cox multivariate regression analysis. P-values less than  
50 0.05 were considered significant.  
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56 Table 1. Primary tumor characteristics.

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<b>Tumor size</b>	
T1	20 (32.8%)
T2	33 (54.1%)
T3	7 (11.5%)
T4	1 (1.6%)
<b>Nodal status</b>	
N0	16 (26.2%)
N1	22 (36.1%)
N2	15 (24.6%)
N3	8 (13.1%)
<b>Histopathology</b>	
Ductal	47 (77.0%)
Lobular	11 (18.0%)
Other	3 (4.9%)
<b>Histopathological grade</b>	
Grade 1	0 (0%)
Grade 2	25 (41.0%)
Grade 3	34 (55.7%)
Unknown	2 (3.3%)
<b>ER expression</b>	
Negative (0 %)	14 (23.0%)
Weak (1-9 %)	2 (3.3%)
Moderate (10-59 %)	6 (9.8%)
High (>59 %)	39 (63.9%)

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<b>PR expression</b>	
Negative (0 %)	22 (36.1%)
Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
<b>HER2 status</b>	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
<b>Ki-67 expression</b>	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
<b>Focality</b>	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
<b>Subtype</b>	
Luminal A-like	13 (21.3%)
Luminal B-like (HER2-negative)	29 (47.5%)
Luminal B-like (HER2-positive)	5 (8.2%)
HER2-positive, non-luminal	3 (4.9%)
Triple-negative	10 (16.4%)
Unknown	1 (1.6%)
<b>The first site of the distant metastasis</b>	



Bone only	17 (27.9%)
Lung only	9 (14.8%)
Liver only	5 (8.2%)
Other	6 (9.6%)
Multiple sites	24 (39.3%)

### **Patient and public involvement**

Patients or public were not involved in the design, conduct, reporting or dissemination of this study.

### **Results**

Sixty-one patients of the originally 594 women ultimately developed distant metastases during the follow-up. Of these, fifty patients died of breast cancer during the follow-up. The median disease-free interval was 39.0 months in the patients who had distant metastases. The median follow-up time starting from the early breast cancer diagnosis was 72.0 months in patients who later developed metastases.

The median follow-up of the patients during their metastatic breast cancer was 18.0 months (mean 30.2 months). The Kaplan-Meier estimate for median survival of the patients with metastatic breast cancer was 77.0 months in those with luminal A-like breast cancers, 29.0 months in those with luminal B-like (HER2-negative) disease and 11.0, 26.0 and 12.0 months in those with HER2-positive, non-luminal, luminal B-like (HER2-positive) and TNBC subtype, respectively.

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3 Patients with metastatic local lymph nodes at the time of definitive surgery displayed poorer  
4 survival outcomes for metastatic disease ( $p = 0.031$ ) (Figure 2). The Kaplan-Meier estimate for  
5 median survival in metastatic disease in lymph node-negative patients was 33.0 months, and in  
6 lymph node-positive patients, it was 19.0 months. Only N0 versus N1-3 classification was  
7 significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes ( $p =$   
8 0.78) were detected.  
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19 Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary  
20 tumor ( $p = 0.011$ ), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors ( $p$   
21 = 0.032) and grade I–II (versus grade III) primary tumors ( $p = 0.012$ ) were associated with better  
22 survival in metastatic disease in univariate analysis. Breast cancer subtype (determined from the  
23 initial surgical samples) also predicted survival with metastatic breast cancer ( $p = 0.0078$ ). Also, the  
24 patients with luminal A-like breast cancer had significantly prolonged survival, when compared to  
25 all other subtypes ( $p = 0.017$ ). Primary tumor size, PR or HER2 expression, the site of the first  
26 metastasis in bone versus elsewhere, disease-free interval ( $\leq 24$  months versus  $> 24$  months) or age  
27 at disease onset were not associated with metastatic disease survival.  
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42 When assessed separately by different biological subtypes, initial lymph node metastases predicted  
43 worse prognosis only in the patients with the luminal A subtype in univariate analysis ( $p = 0.019$ ),  
44 but the small sample size of each subgroup limited the reliability of this analysis (data not shown).  
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51 In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis  
52 predicted poorer survival overall (HR, 2.17; 95% CI, 1.09-4.31;  $p = 0.027$ ) when tumor size (T1  
53 versus T2-4) (HR, 1.33; 95% CI, 0.71-2.47;  $p = 0.37$ ) and ER status (negative versus positive) (HR  
54 2.16; 95% CI 1.14-4.10;  $p = 0.018$ ) were included in the analysis. The proportional hazards  
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3 assumption was met in the analysis. Breast cancer subtype, Ki-67 expression or grade did not  
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5 remain significant prognostic factors after multivariate analysis.  
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## 10 **Discussion**

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14 As the main observation, we report in this prospectively collected and contemporary cohort from a  
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16 Finnish University Hospital that the presence of local lymph node metastasis at the time of early  
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18 breast cancer surgery predicted short survival in subsequent metastatic breast cancer. Our results  
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20 also supported previous results of ER negativity in primary breast cancer as an adverse prognostic  
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22 factor for disease recurrence.  
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28 The most established prognostic factors of better outcome in metastatic breast cancer include ER  
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30 positivity, long disease-free interval (usually defined as at least 2 years), low number of metastatic  
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32 sites and bone-only localization of metastases [7-14, 18-20]. HER2 appears to no longer represent a  
33  
34 prognostic factor in the era of targeted treatments, and prognostic factors also slightly differ  
35  
36 between HER2-positive and HER2-negative patients [21, 22]. Emerging metastatic breast cancer  
37  
38 prognostic factors include circulating tumor cells, gene expression panels, circulating tumor  
39  
40 markers and miRNAs; however, they have not yet been sufficiently validated [23-26]. Whereas ER  
41  
42 status, a lengthy disease-free interval and metastatic load are established and obvious prognostic  
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44 factors for metastatic breast cancer, the presence of lymph node metastases at the time of initial  
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46 diagnosis has not been widely studied in metastatic breast cancer, although it is the strongest  
47  
48 prognostic factor in early breast cancer. In the current study, we concentrated solely on primary  
49  
50 breast cancer characteristics, and we did not assess other characteristics, such as disease-free  
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52 interval, metastasis load or metastasis location as prognostic factors.  
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3 Some previous studies with mostly retrospective cohort settings and outdated treatment modalities  
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5 have reported the initial nodal status as a prognostic factor in metastatic breast cancer, whereas  
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7 others have not found such an association [7, 27-29]. In the pioneer work of Clark et al., nodal  
8  
9 involvement at time of initial diagnosis was associated with shorter survival [12]. Another  
10  
11 retrospective single-institute study also concluded that lymph node involvement at primary  
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13 diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients  
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15 were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish  
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17 retrospective registry study suggested that the initial lymph node status should be part of the  
18  
19 prognostic index in recurrent metastatic breast cancer [31]. In addition to considerable change in the  
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21 oncological treatments of breast cancer during the last decades, also surgical techniques, especially  
22  
23 axillary procedures have developed considerably. The current results from the prospective data with  
24  
25 modern treatments thus support and confirm earlier results.  
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33 In our study, any metastasis found in local lymph nodes at the time of definitive surgery was  
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35 associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median  
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37 survival was prolonged from 19–33 months in patients without lymph node metastases at the time  
38  
39 of initial diagnosis. Although lymph node metastases in general is associated with other factors of  
40  
41 poor prognosis, our study suggested that this result was independent of tumor size and ER  
42  
43 expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it  
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45 can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent  
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47 paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions  
48  
49 suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for  
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51 predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per*  
52  
53 *se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually  
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55 metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary  
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3 lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these  
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5 issues continues [36].  
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10 It has to be emphasized that all our patients had recurrent breast cancer, and our material did not  
11  
12 include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the  
13  
14 material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast  
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16 cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless,  
17  
18 the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer,  
19  
20 and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9,  
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22 37]. As an other limitation, we were unable to address the results separately in subgroups, for  
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24 example according to biological subtypes, due to relatively low number of patients with metastatic  
25  
26 breast cancer. On the other hand, our study was based on a prospective cohort from a university  
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28 clinic, and the patients were treated with up-to-date surgical and oncological treatment modalities.  
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35 Our results confirmed that ER negativity in primary tumor was associated with short survival for  
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37 metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but  
38  
39 also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER  
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41 negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12,  
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43 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER  
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45 status is also a predictor of poor prognosis [38]. Most previous studies have divided metastatic  
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47 breast cancers only to three subgroups: ER/PR-positive, HER2-positive and TNBC. We used the  
48  
49 widely recognized ESMO guidelines for subtyping our cases. Although the number of patients in  
50  
51 each subgroup were rather limited, the patients with slowly proliferating, ER-responsive luminal A-  
52  
53 like breast cancers still had significantly prolonged survival in metastatic breast cancer compared to  
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3 other subtypes. TNBC has the worst outcome of all subtypes in metastatic breast cancer, a finding  
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5 which was mirrored in our study [39].  
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10 Predicting the course of metastatic breast cancer is of primary importance in clinical practice;  
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12 however, its status as a highly heterogenous disease at both the inpatient and outpatient levels  
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14 makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer  
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16 guidelines recommend starting treatment with chemotherapy or even with a combination  
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18 chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing  
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20 ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic  
21  
22 breast cancer, such as initial nodal status, could be confirmed, these patients should receive more  
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24 aggressive first-line metastatic breast cancer therapy.  
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30 In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse  
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32 prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial  
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34 lymph node status may be a prognostic factor for metastatic disease course. Future studies should  
35  
36 also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute  
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38 number of metastatic lymph nodes, which were not addressed in our material. More research is also  
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40 clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or  
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42 whether they are purely an indicator of aggressive disease.  
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### 49 **Contributorship statement**

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52

53 All authors contributed to the study design and conception. AJu initiated the collection of the  
54  
55 prospective dataset. PK, AJa and NR were responsible for assessing statistical analyses. PK was a  
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2  
3 major contributor in writing the manuscript. All authors provided comments on drafts of the  
4 manuscript. All authors read and approved the final manuscript.  
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### 10 **Competing interests**

11  
12  
13  
14 The authors report no competing interests.  
15  
16  
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### 19 **Funding**

20  
21  
22  
23 There are no funders to report for this submission.  
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### 28 **Data sharing statement**

29  
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32 Data are available upon reasonable request.  
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### 37 **Figure legends**

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42 Figure 1. Flow chart of the study participants.  
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47 Figure 2. Associations between primary tumor properties and survival in metastatic breast cancer.

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49 In multivariate analysis, only ER expression and initial nodal status remained as significant factors.  
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### 58 **References**

- 1  
2  
3 1 Bray F, Ferlay J, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and  
4 mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.  
5
- 6  
7 2 Cardoso F, Senkus E, Costa A, et al. 4th ESO-ESMO International Consensus Guidelines for  
8 Advanced Breast Cancer (ABC 4)dagger. *Ann Oncol* 2018;29:1634-57.  
9
- 10  
11 3 Cardoso F, Spence D, Mertz S, Corneliussen-James D, Sabelko K, Gralow J, et al. Global analysis  
12 of advanced/metastatic breast cancer: Decade report (2005-2015). *Breast* 2018;39:131-8.  
13
- 14  
15 4 Allemani C, Weir HK, Carreira H, et al. Global surveillance of cancer survival 1995-2009:  
16 analysis of individual data for 25,676,887 patients from 279 population-based registries in 67  
17 countries (CONCORD-2). *Lancet* 2015;385:977-1010.  
18
- 19  
20 5 Weigelt B, Peterse JL, van 't Veer, LJ. Breast cancer metastasis: markers and models. *Nat Rev*  
21 *Cancer* 2005;5:591-602.  
22
- 23  
24 6 Early Breast Cancer Trialists' Collaborative Group, (EBCTCG), Peto R, Davies C, Godwin J,  
25 Gray R, Pan HC, et al. Comparisons between different polychemotherapy regimens for early breast  
26 cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials.  
27 *Lancet* 2012;379:432-44.  
28
- 29  
30 7 Chang J, Clark GM, Allred DC, et al. Survival of patients with metastatic breast carcinoma:  
31 importance of prognostic markers of the primary tumor. *Cancer* 2003;97:545-53.  
32
- 33  
34 8 Regierer AC, Wolters R, Ufen M, et al. An internally and externally validated prognostic score  
35 for metastatic breast cancer: analysis of 2269 patients. *Ann Oncol* 2014;25:633-8.  
36
- 37  
38 9 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast  
39 cancer. *Ann Oncol* 2008;19:2012-9.  
40
- 41  
42 10 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-  
43 free interval is important. *Hum Pathol* 2017;70:70-6.  
44
- 45  
46 11 Stuart-Harris R, Shadbolt B, Palmqvist C, et al. The prognostic significance of single hormone  
47 receptor positive metastatic breast cancer: an analysis of three randomised phase III trials of  
48 aromatase inhibitors. *Breast* 2009;18:351-5.  
49
- 50  
51 12 Clark GM, Sledge GW, Jr, Osborne CK, et al. Survival from first recurrence: relative importance  
52 of prognostic factors in 1,015 breast cancer patients. *J Clin Oncol* 1987;5(1):55-61.  
53
- 54  
55 13 Shen T, Gao C, Zhang K, et al. Prognostic outcomes in advanced breast cancer: the metastasis-  
56 free interval is important. *Hum Pathol* 2017;70:70-6.  
57
- 58  
59 14 Lobbezoo DJA, van Kampen, RJW., Voogd AC, et al. Prognosis of metastatic breast cancer: are  
60 there differences between patients with de novo and recurrent metastatic breast cancer? *Br J Cancer*  
2015;112:1445-51.
- 15 Senkus E, Kyriakides S, Ohno S, et al. Primary breast cancer: ESMO Clinical Practice  
Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2015;26 Suppl 5:8.



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59  
60

16 Karihtala P, Mäntyniemi A, Kang SW, et al. Peroxiredoxins in breast carcinoma. *Clin Cancer Res* 2003;9:3418-24.

17 Isola J, Tanner M, Forsyth A, et al. Interlaboratory comparison of HER-2 oncogene amplification as detected by chromogenic and fluorescence in situ hybridization. *Clin Cancer Res* 2004;10:4793-8.

18 Yamamoto N, Watanabe T, Katsumata N, et al. Construction and validation of a practical prognostic index for patients with metastatic breast cancer. *J Clin Oncol* 1998;16:2401-8.

19 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast cancer patients: analysis of deceased patients. *Rad Oncol J* 2013;31:222-7.

20 Khanfir A, Lahiani F, Bouzguenda R, et al. Prognostic factors and survival in metastatic breast cancer: A single institution experience. *Rep Pract Oncol Radiother* 2013;18:127-32.

21 Hopkins AM, Rowland A, McKinnon RA, et al. Predictors of Long-Term Disease Control and Survival for HER2-Positive Advanced Breast Cancer Patients Treated With Pertuzumab, Trastuzumab, and Docetaxel. *Front Oncol* 2019;9:789.

22 Dawood S, Broglio K, Buzdar AU, et al. Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. *J Clin Oncol* 2010;28:92-8.

23 King TA, Lyman JP, Gonen M, et al. Prognostic Impact of 21-Gene Recurrence Score in Patients With Stage IV Breast Cancer: TBCRC 013. *J Clin Oncol* 2016 Jul 10;34(20):2359-65.

24 Prat A, Cheang MC, Galvan P, et al. Prognostic Value of Intrinsic Subtypes in Hormone Receptor-Positive Metastatic Breast Cancer Treated With Letrozole With or Without Lapatinib. *JAMA Oncol* 2016;2:1287-94.

25 Papadaki C, Stoupis G, Tsalikis L, et al. Circulating miRNAs as a marker of metastatic disease and prognostic factor in metastatic breast cancer. *Oncotarget* 2019;10:966-81.

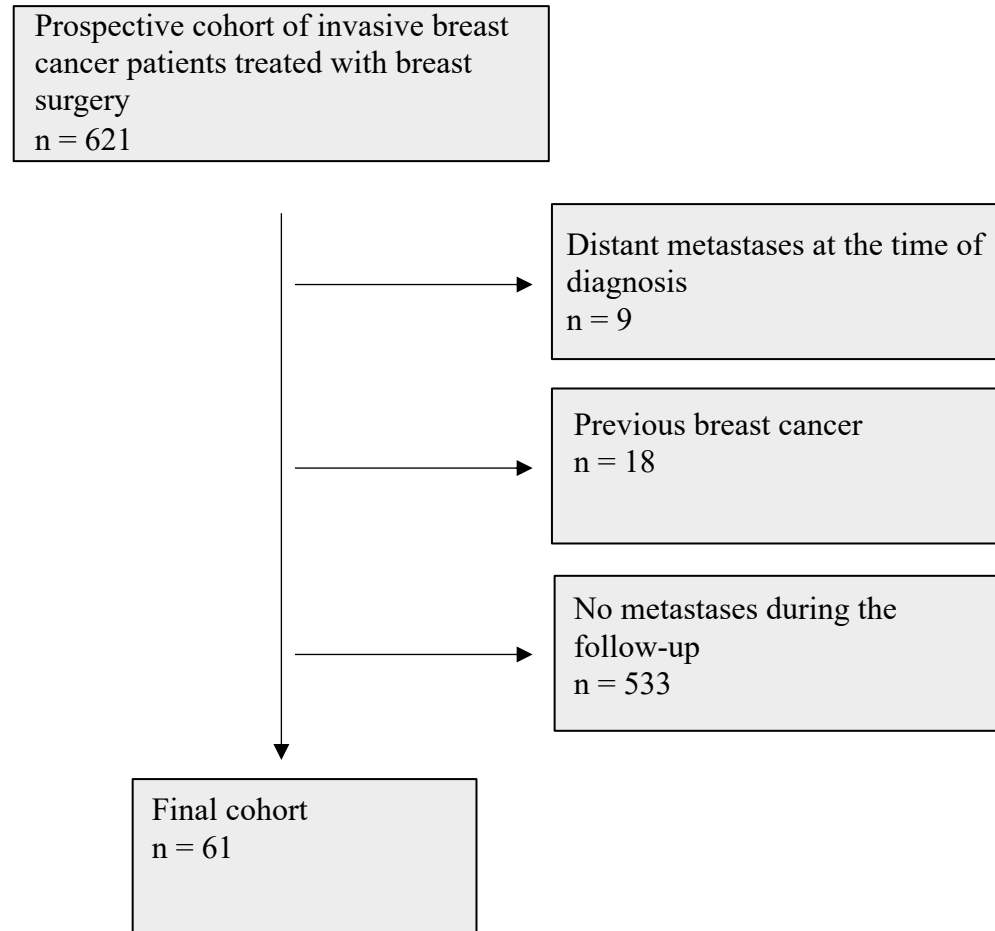
26 Van Poznak C, Somerfield MR, Bast RC, et al. Use of Biomarkers to Guide Decisions on Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol* 2015;33(24):2695-704.

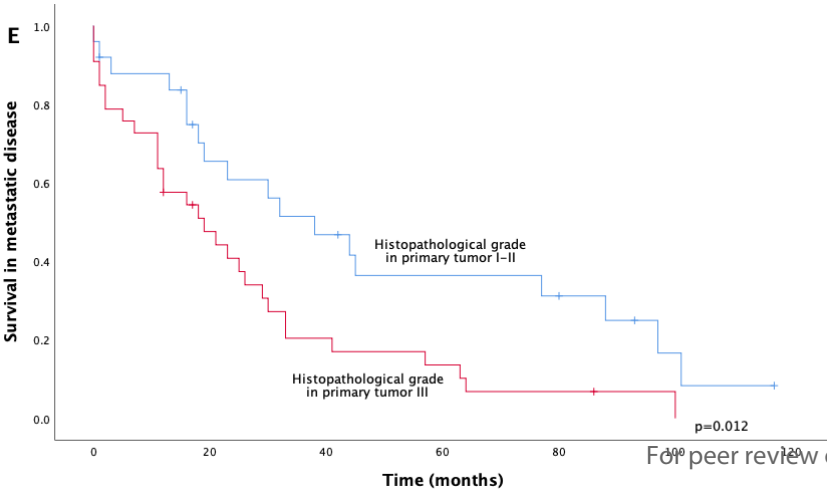
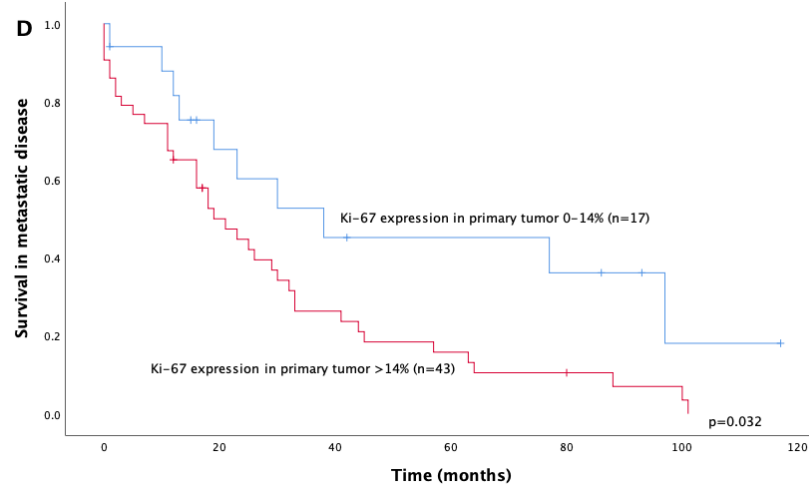
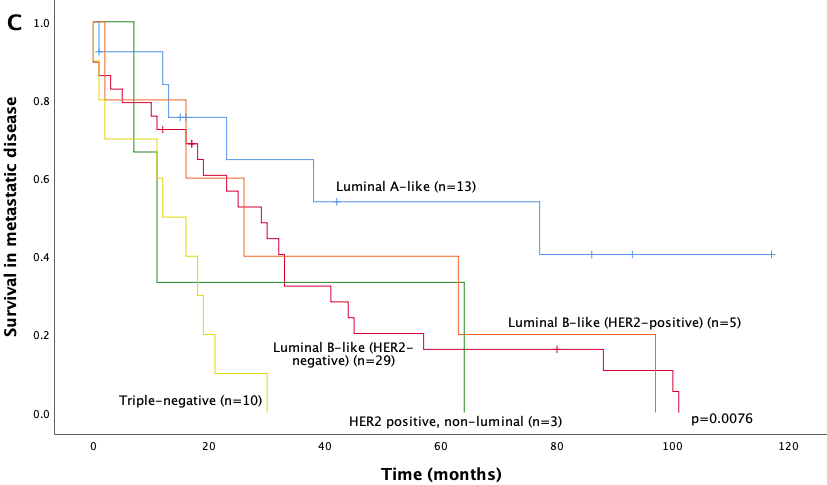
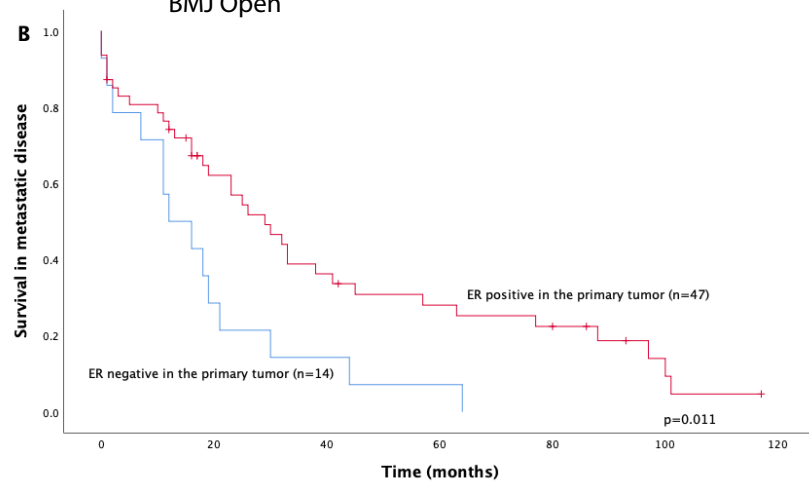
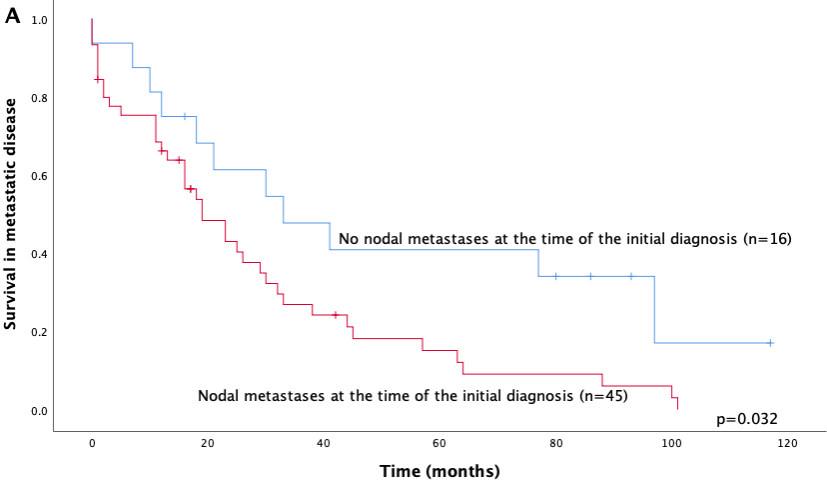
27 Largillier R, Ferrero J, Doyen J, et al. Prognostic factors in 1,038 women with metastatic breast cancer. *Ann Oncol* 2008;19:2012-9.

28 Insa A, Lluch A, Prosper F, et al. Prognostic factors predicting survival from first recurrence in patients with metastatic breast cancer: analysis of 439 patients. *Breast Cancer Res Treat* 1999;56(1):67-78.

29 Tsuji W, Teramukai S, Ueno M, et al. Prognostic factors for survival after first recurrence in breast cancer: a retrospective analysis of 252 recurrent cases at a single institution. *Breast Cancer* 2014;21:86-95.

- 1  
2  
3 30 Rack B, Janni W, Gerber B, et al. Patients with recurrent breast cancer: does the primary axillary  
4 lymph node status predict more aggressive tumor progression? *Breast Cancer Res Treat*  
5 2003;82(2):83-92.  
6  
7  
8 31 Puente J, Lopez-Tarruella S, Ruiz A, et al. Practical prognostic index for patients with metastatic  
9 recurrent breast cancer: retrospective analysis of 2,322 patients from the GEICAM Spanish El  
10 Alamo Register. *Breast Cancer Res Treat* 2010;122:591-600.  
11  
12 32 Gibert-Ramos A, Lopez C, Bosch R, et al. Immune response profile of primary tumour, sentinel  
13 and non-sentinel axillary lymph nodes related to metastasis in breast cancer: an  
14 immunohistochemical point of view. *Histochem Cell Biol* 2019;152:177-93.  
15  
16 33 Ullah I, Karthik G, Alkodsi A, et al. Evolutionary history of metastatic breast cancer reveals  
17 minimal seeding from axillary lymph nodes. *J Clin Invest* 2018;128:1355-70.  
18  
19 34 Klein CA. Selection and adaptation during metastatic cancer progression. *Nature* 2013 Sep  
20 19;501:365-72.  
21  
22 35 Engel J, Emeny RT, Hölzel D. Positive lymph nodes do not metastasize. *Cancer Metastasis Rev*  
23 2012;31:235-46.  
24  
25 36 Barry P, Vatsiou A, Spiteri I, et al. The Spatiotemporal Evolution of Lymph Node Spread in  
26 Early Breast Cancer. *Clin Cancer Res* 2018;24:4763-70.  
27  
28 37 Kim H, Choi DH, Park W, et al. Prognostic factors for survivals from first relapse in breast  
29 cancer patients: analysis of deceased patients. *Radiat Oncol J* 2013;31:222-7.  
30  
31 38 Woo JW, Chung YR, Ahn S, et al. Changes in Biomarker Status in Metastatic Breast Cancer and  
32 Their Prognostic Value. *J Breast Cancer* 2019;22:439-52.  
33  
34 39 Fietz T, Tesch H, Rauh J, et al. Palliative systemic therapy and overall survival of 1,395 patients  
35 with advanced breast cancer - Results from the prospective German TMK cohort study. *Breast*  
36 2017;34:122-30.  
37  
38 40 Januskeviciene I, Petrikaite V. Heterogeneity of breast cancer: The importance of interaction  
39 between different tumor cell populations. *Life Sci* 2019;239:117009.  
40  
41 41 Breast Cancer [Internet].; 2019 [updated Sep 6,; cited Jan 12, 2019]. Available from:  
42  
43 [https://www.nccn.org/professionals/physician\\_gls/pdf/breast.pdf](https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf).  
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## STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1	a prospective single-center study
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
<b>Introduction</b>				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses		
<b>Methods</b>				
Study design	4	Present key elements of study design early in the paper	4	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case		
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3, 4	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4	
Bias	9	Describe any efforts to address potential sources of bias	5, 11	
Study size	10	Explain how the study size was arrived at	4	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4, 8, 9
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	5
		(b) Describe any methods used to examine subgroups and interactions	4, 5
		(c) Explain how missing data were addressed	5-7
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	
		(e) Describe any sensitivity analyses	
<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	3
		(b) Give reasons for non-participation at each stage	3
		(c) Consider use of a flow diagram	As Figure
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-7
		(b) Indicate number of participants with missing data for each variable of interest	5-7
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	8
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(b) Report category boundaries when continuous variables were categorized	9
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	2, 3, 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-12
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-12
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).