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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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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

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. Patients with previous breast cancer or distant metastases at the time of diagnosis were excluded from the cohort. 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 ≥ 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 immunohistochemistry as previously described [16]. HER2 expression was studied using immunohistochemistry and chromogenic in situ hybridization (CISH) to confirm positive results. A positive result of six or more gene copies in CISH was considered HER2-positive [17] .

Ethical considerations

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

Statistical analyses

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

Table 1. Primary tumor characteristics.

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

Moderate (10-59 %)	6 (9.8%)
High (>59 %)	39 (63.9%)
PR expression	
Negative (0 %)	22 (36.1%)
Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
HER2 status	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
Ki-67 expression	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
Focality	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
Subtype	
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%)
The first site of the distant metastasis	
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

significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes (p = 0.78) were detected.

Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary tumor (p = 0.011), breast cancer subtype (determined from the initial surgical samples) (p = 0.0078), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors (p = 0.032) and grade I–II (versus grade III) primary tumors (p = 0.012) were associated with better survival in metastatic disease in univariate analysis. Primary tumor size, PR or HER2 expression or age at disease onset were not associated with metastatic disease survival.

When assessed separately by different biological subtypes, initial lymph node metastases predicted worse prognosis only in the patients with the luminal A subtype in univariate analysis (p = 0.019), but the small sample size of each subgroup limited the reliability of this analysis (data not shown).

In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis predicted poorer survival overall (HR, 2.17; 95% CI, 1.09-4.31; p = 0.027) when tumor size (T1 versus T2-4) (HR, 1.33; 95% CI, 0.71-2.47; p = 0.37) and ER status (negative versus positive) (HR 2.16; 95% CI 1.14-4.10; p = 0.018) were included in the analysis. Breast cancer subtype, Ki-67 expression or grade did not remain significant prognostic factors after multivariate analysis.

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 involvement at time of initial diagnosis was associated with shorter survival [12]. Another retrospective single-institute study also concluded that lymph node involvement at primary diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish

retrospective registry study suggested that the initial lymph node status should be part of the prognostic index in recurrent metastatic breast cancer [31].

In our study, any metastasis found in local lymph nodes at the time of definitive surgery was associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median survival was prolonged from 19–33 months in patients without lymph node metastases at the time of initial diagnosis. Although lymph node metastases in general is associated with other factors of poor prognosis, our study suggested that this result was independent of tumor size and ER expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these issues continues [36] .

It has to be emphasized that all our patients had recurrent breast cancer, and our material did not include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless, the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer, and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9,

37]. Our study was based on a prospective cohort from a university clinic, and the patients were treated with up-to-date surgical and oncological treatment modalities.

Our results confirmed that ER negativity in primary tumor was associated with short survival for metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12, 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER status is also a predictor of poor prognosis [38]. TNBC has the worst outcome of all subtypes in metastatic breast cancer, a finding which was mirrored in our study [39].

Predicting the course of metastatic breast cancer is of primary importance in clinical practice; however, its status as a highly heterogenous disease at both the intrapatient and interpatient levels makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer guidelines recommend starting treatment with chemotherapy or even with a combination chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic breast cancer, such as initial nodal status, could be confirmed, these patients should receive more aggressive first-line metastatic breast cancer therapy.

In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial lymph node status may be a prognostic factor for metastatic disease course. Future studies should also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute number of metastatic lymph nodes, which were not addressed in our material. More research is also

clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or whether they are purely an indicator of aggressive disease.

Contributorship statement

All authors contributed to the study design and conception. AJu initiated the collection of the prospective dataset. PK, AJä and NR were responsible for assessing statistical analyses. PK was a major contributor in writing the manuscript. All authors provided comments on drafts of the manuscript. All authors read and approved the final manuscript.

Data sharing statement

No additional data available.

Figure legends

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

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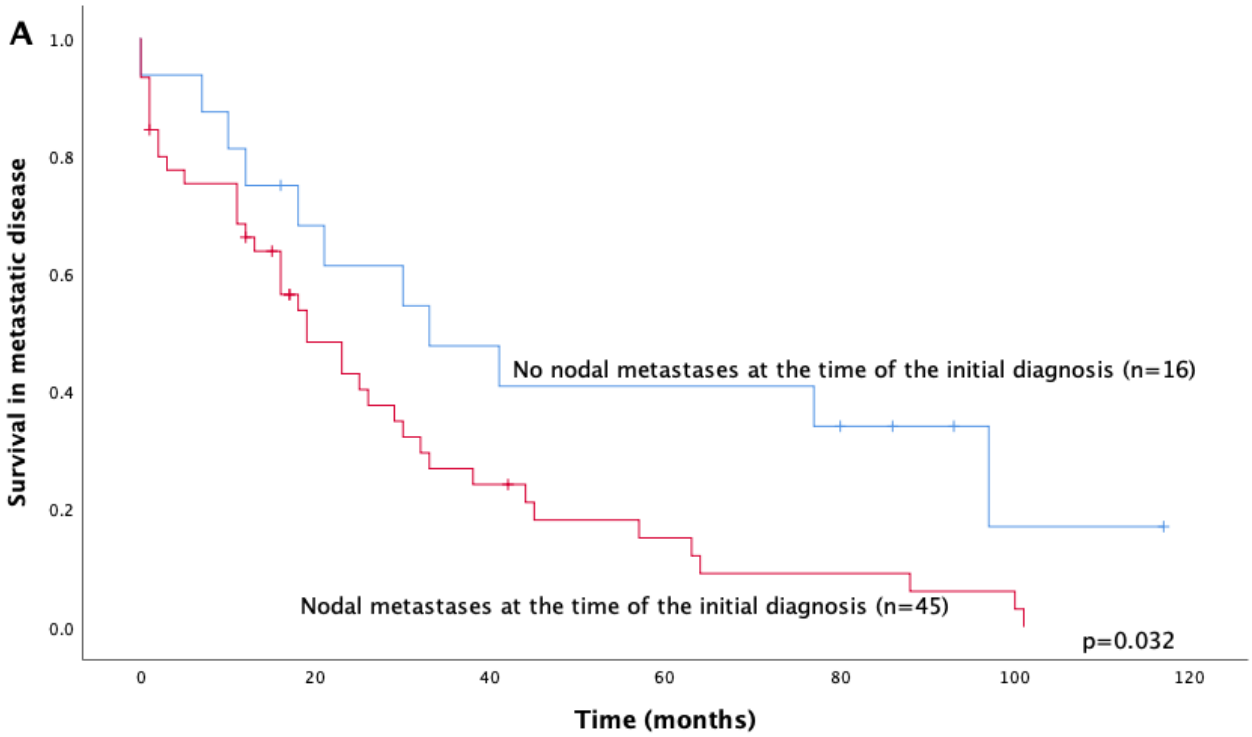
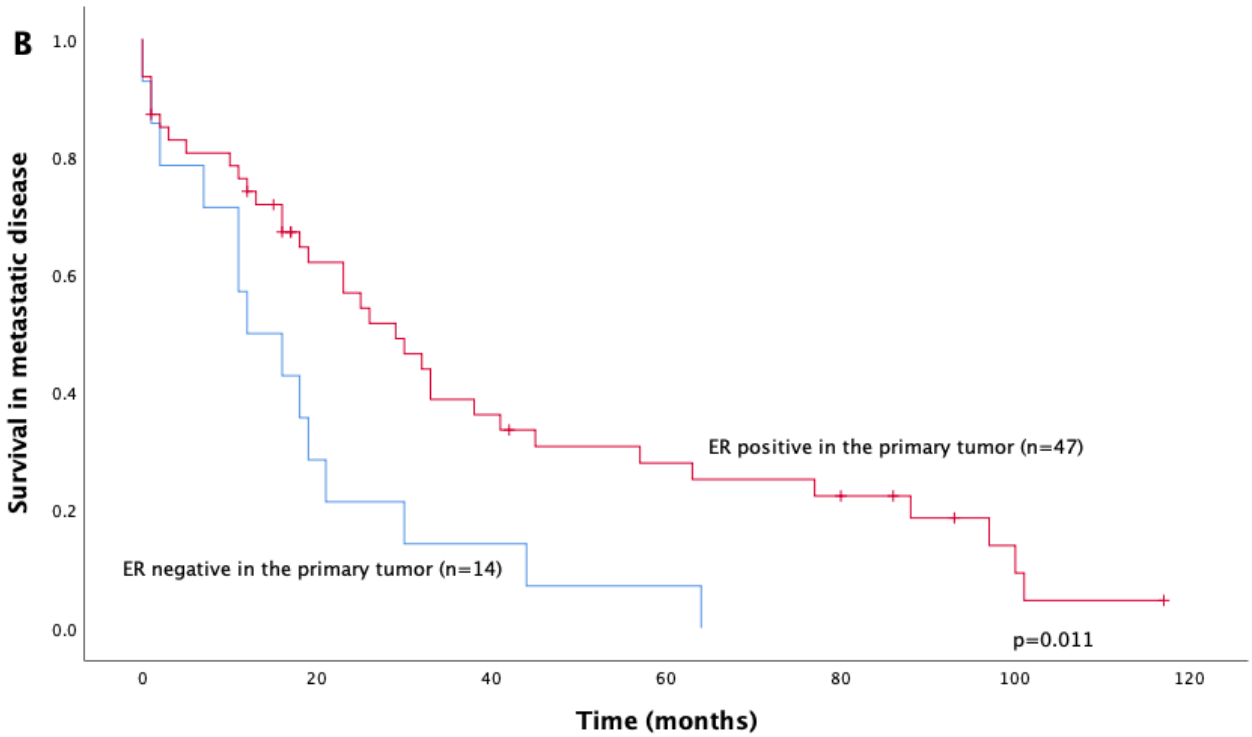
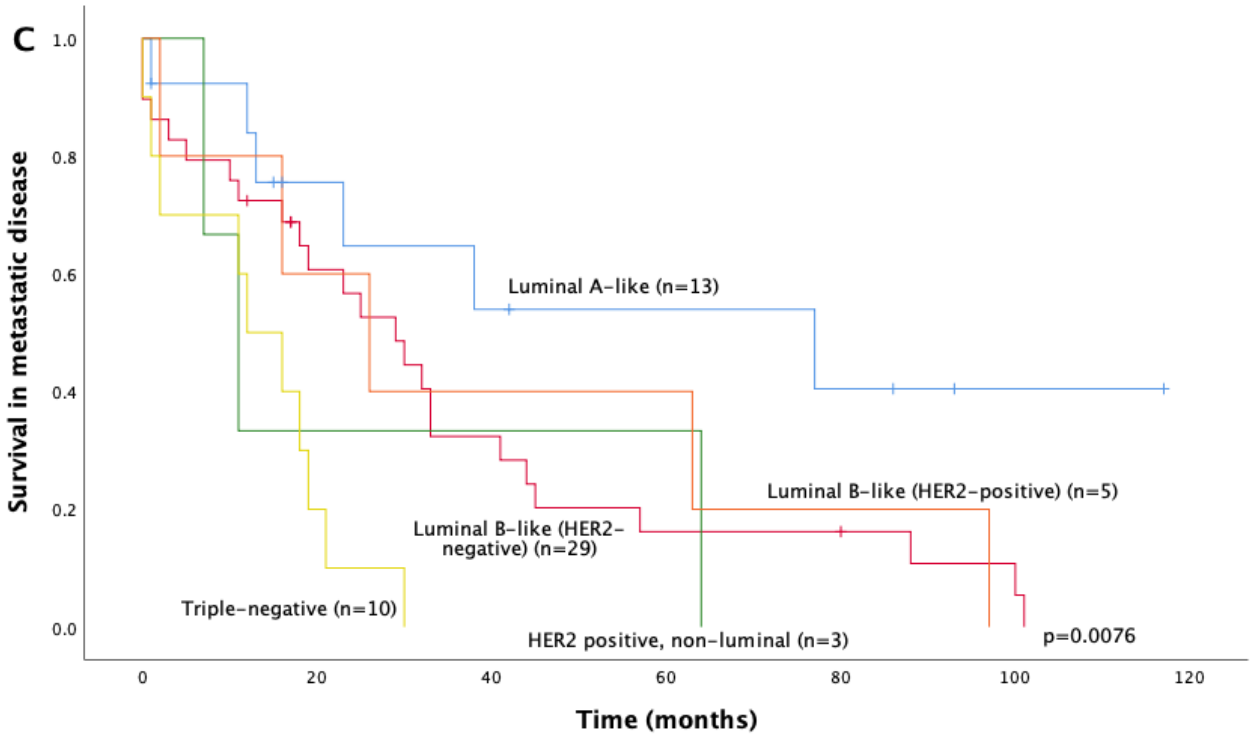
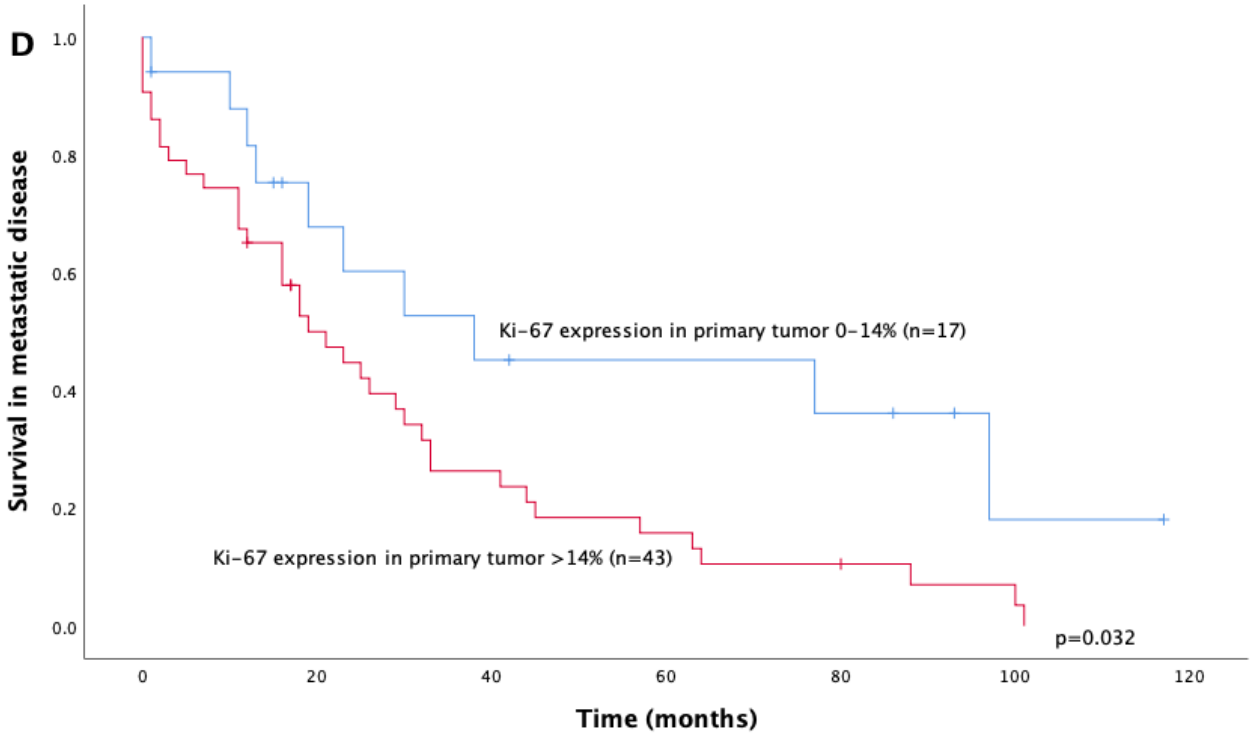
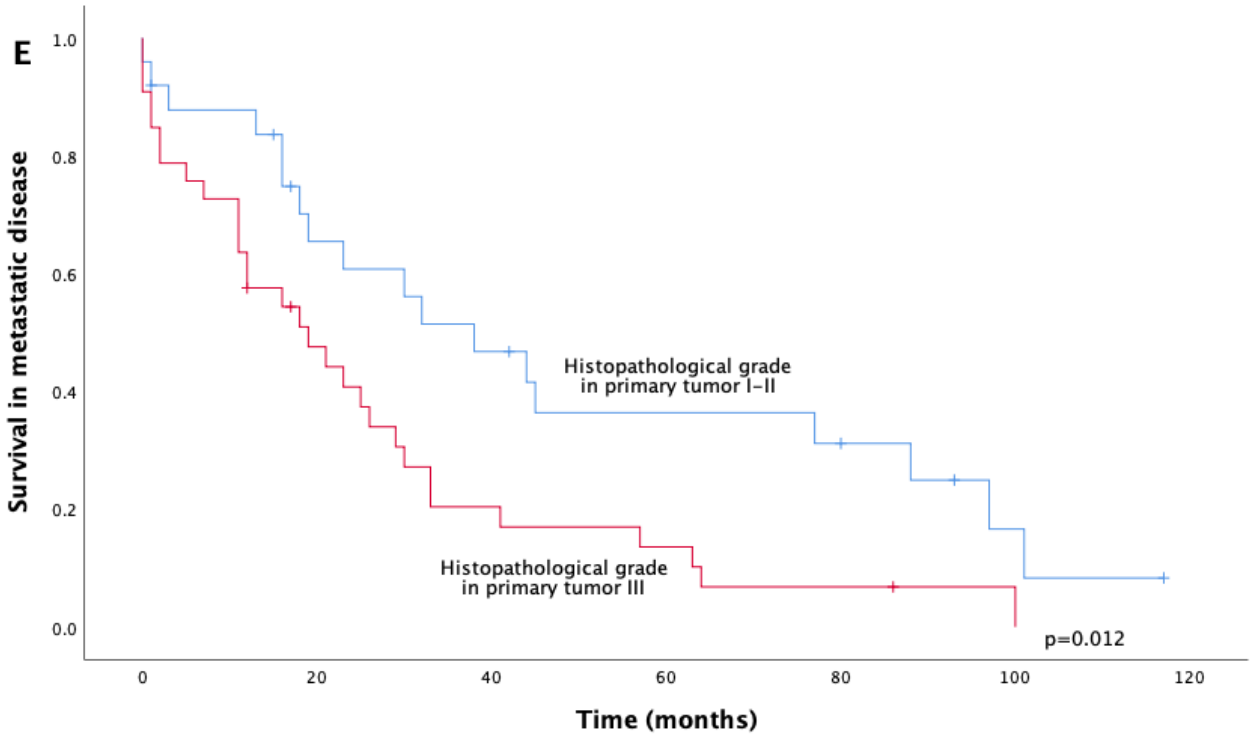

A
Survival in metastatic disease
1.0
0.8
0.6
0.4
0.2
0.0
No nodal metastases at the time of the initial diagnosis (n=16)
Nodal metastases at the time of the initial diagnosis (n=45)
p=0.032
0
20
40
60
80
100
120
Time (months)


B
Survival in metastatic disease
1.0
0.8
0.6
0.4
0.2
0.0
ER positive in the primary tumor (n=47)
ER negative in the primary tumor (n=14)
p=0.011
0
20
40
60
80
100
120
Time (months)


C
Survival in metastatic disease
1.0
0.8
0.6
0.4
0.2
0.0
Luminal A-like (n=13)
Luminal B-like (HER2-positive) (n=5)
Luminal B-like (HER2-negative) (n=29)
Triple-negative (n=10)
HER2 positive, non-luminal (n=3)
p=0.0076
0
20
40
60
80
100
120
Time (months)


D
Survival in metastatic disease
1.0
0.8
0.6
0.4
0.2
0.0
Ki-67 expression in primary tumor 0-14% (n=17)
Ki-67 expression in primary tumor >14% (n=43)
p=0.032
0
20
40
60
80
100
120
Time (months)



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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 ≥ 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

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

Ethical considerations

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

Statistical analyses

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

Table 1. Primary tumor characteristics.

	N (%)
Tumor size	
T1	20 (32.8%)

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

Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
HER2 status	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
Ki-67 expression	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
Focality	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
Subtype	
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%)
The first site of the distant metastasis	
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

lymph node-positive patients, it was 19.0 months. Only N0 versus N1-3 classification was significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes (p = 0.78) were detected.

Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary tumor (p = 0.011), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors (p = 0.032) and grade I–II (versus grade III) primary tumors (p = 0.012) were associated with better survival in metastatic disease in univariate analysis. Breast cancer subtype (determined from the initial surgical samples) also predicted survival with metastatic breast cancer (p = 0.0078) (Figure 2C), Also, the patients with luminal A-like breast cancer had significantly prolonged survival, when compared to all other subtypes (p = 0.017). Primary tumor size, PR or HER2 expression, the site of the first metastasis in bone versus elsewhere, disease-free interval (≤ 24 months versus > 24 months) or age at disease onset were not associated with metastatic disease survival.

When assessed separately by different biological subtypes, initial lymph node metastases predicted worse prognosis only in the patients with the luminal A subtype in univariate analysis (p = 0.019), but the small sample size of each subgroup limited the reliability of this analysis (data not shown).

In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis predicted poorer survival overall (HR, 2.17; 95% CI, 1.09-4.31; p = 0.027) when tumor size (T1 versus T2-4) (HR, 1.33; 95% CI, 0.71-2.47; p = 0.37) and ER status (negative versus positive) (HR 2.16; 95% CI 1.14-4.10; p = 0.018) were included in the analysis. Breast cancer subtype, Ki-67 expression or grade did not remain significant prognostic factors after multivariate analysis.

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 involvement at time of initial diagnosis was associated with shorter survival [12]. Another

retrospective single-institute study also concluded that lymph node involvement at primary diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish retrospective registry study suggested that the initial lymph node status should be part of the prognostic index in recurrent metastatic breast cancer [31]. In addition to considerable change in the oncological treatments of breast cancer during the last decades, also surgical techniques, especially axillary procedures have developed considerably. The current results from the prospective data with modern treatments thus support and confirm earlier results.

In our study, any metastasis found in local lymph nodes at the time of definitive surgery was associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median survival was prolonged from 19–33 months in patients without lymph node metastases at the time of initial diagnosis. Although lymph node metastases in general is associated with other factors of poor prognosis, our study suggested that this result was independent of tumor size and ER expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these issues continues [36].

It has to be emphasized that all our patients had recurrent breast cancer, and our material did not include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless, the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer, and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9, 37]. As an other limitation, we were unable to address the results separately in subgroups, for example according to biological subtypes, due to relatively low number of patients with metastatic breast cancer. On the other hand, our study was based on a prospective cohort from a university clinic, and the patients were treated with up-to-date surgical and oncological treatment modalities.

Our results confirmed that ER negativity in primary tumor was associated with short survival for metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12, 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER status is also a predictor of poor prognosis [38]. Most previous studies have divided metastatic breast cancers only to three subgroups: ER/PR-positive, HER2-positive and TNBC. We used the widely recognized ESMO guidelines for subtyping our cases. Although the number of patients in each subgroup were rather limited, the patients with slowly proliferating, ER-responsive luminal A-like breast cancers still had significantly prolonged survival in metastatic breast cancer compared to other subtypes. TNBC has the worst outcome of all subtypes in metastatic breast cancer, a finding which was mirrored in our study [39].

Predicting the course of metastatic breast cancer is of primary importance in clinical practice; however, its status as a highly heterogenous disease at both the intrapatient and interpatient levels makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer guidelines recommend starting treatment with chemotherapy or even with a combination chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic breast cancer, such as initial nodal status, could be confirmed, these patients should receive more aggressive first-line metastatic breast cancer therapy.

In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial lymph node status may be a prognostic factor for metastatic disease course. Future studies should also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute number of metastatic lymph nodes, which were not addressed in our material. More research is also clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or whether they are purely an indicator of aggressive disease.

Contributorship statement

All authors contributed to the study design and conception. AJu initiated the collection of the prospective dataset. PK, AJä and NR were responsible for assessing statistical analyses. PK was a major contributor in writing the manuscript. All authors provided comments on drafts of the manuscript. All authors read and approved the final manuscript.

Data sharing statement

No additional data available.

Figure legends

Figure 1. Flow chart of the study participants.

Figure 2. Associations between primary tumor properties and survival in metastatic breast cancer.

In multivariate analysis, only ER expression and initial nodal status remained as significant factors.

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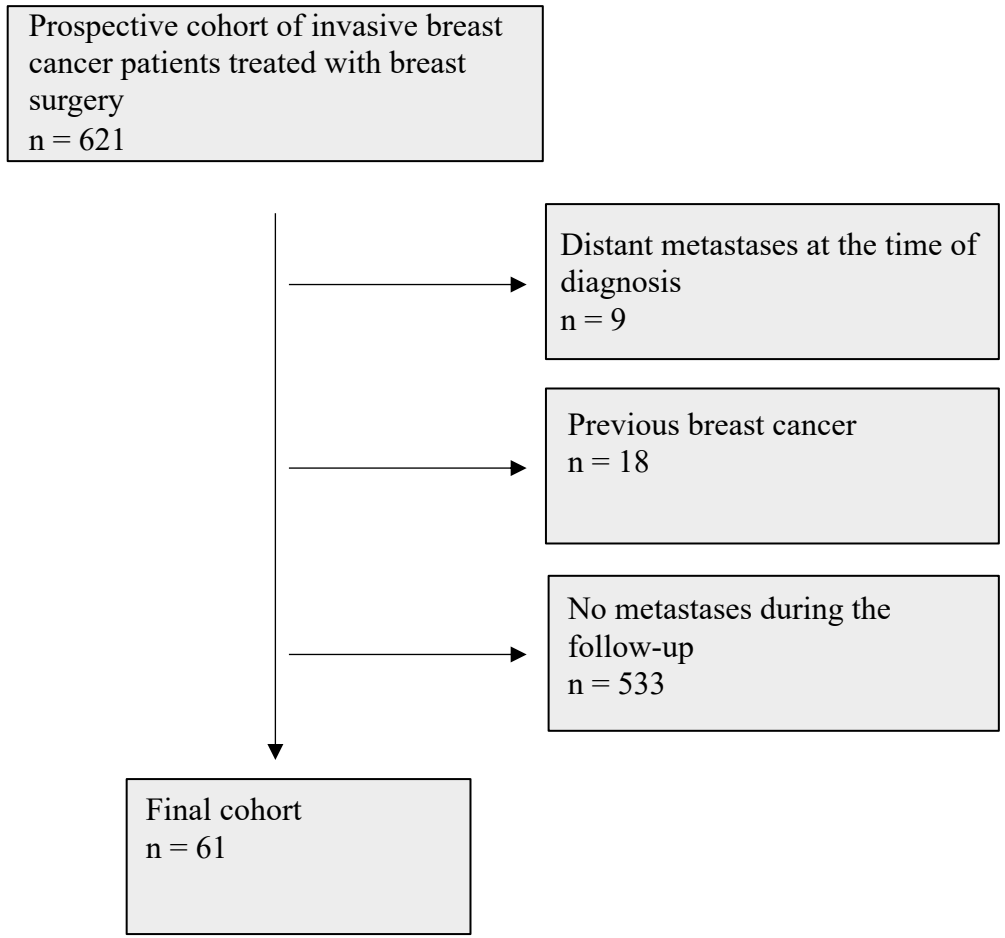
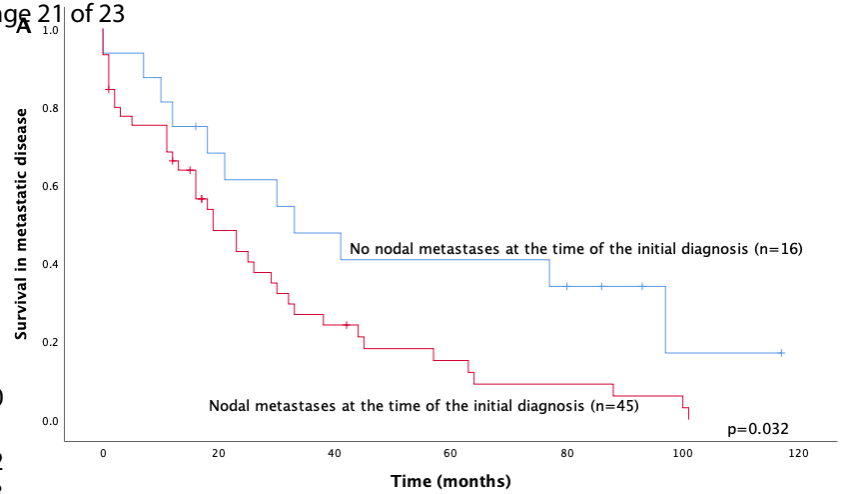
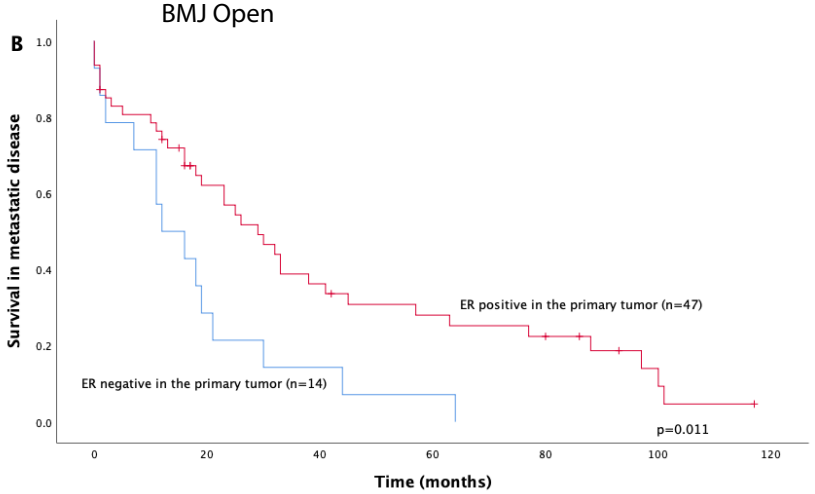
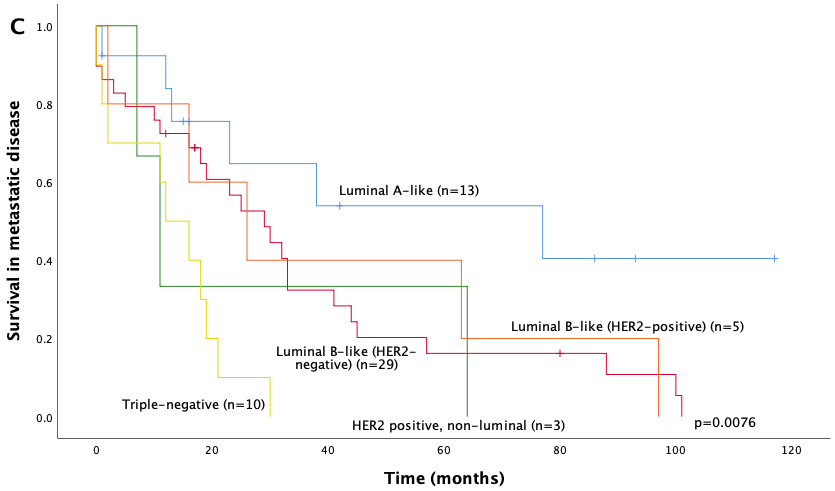
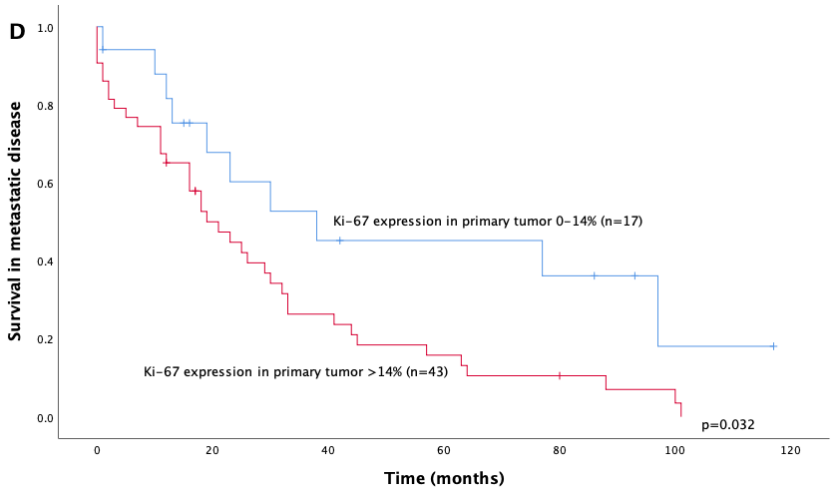
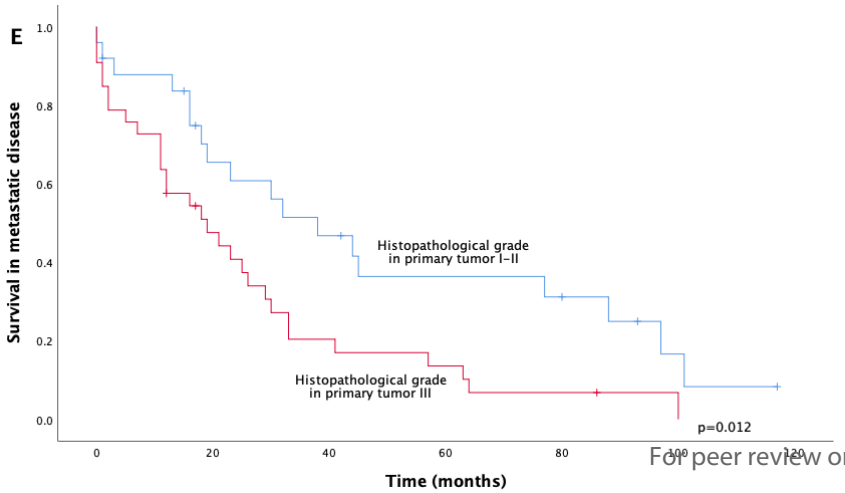

Prospective cohort of invasive breast cancer patients treated with breast surgery
n = 621
Distant metastases at the time of diagnosis
n = 9
Previous breast cancer
n = 18
No metastases during the follow-up
n = 533
Final cohort
n = 61











STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	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	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses		
Methods				
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3	
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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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	
Discussion			
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
Other information			
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.

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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 ≥ 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

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

Ethical considerations

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

Statistical analyses

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

Table 1. Primary tumor characteristics.

	N (%)
Tumor size	
T1	20 (32.8%)

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

Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
HER2 status	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
Ki-67 expression	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
Focality	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
Subtype	
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%)
The first site of the distant metastasis	
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

lymph node-positive patients, it was 19.0 months. Only N0 versus N1-3 classification was significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes (p = 0.78) were detected.

Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary tumor (p = 0.011), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors (p = 0.032) and grade I–II (versus grade III) primary tumors (p = 0.012) were associated with better survival in metastatic disease in univariate analysis. Breast cancer subtype (determined from the initial surgical samples) also predicted survival with metastatic breast cancer (p = 0.0078) (Figure 2C), Also, the patients with luminal A-like breast cancer had significantly prolonged survival, when compared to all other subtypes (p = 0.017). Primary tumor size, PR or HER2 expression, the site of the first metastasis in bone versus elsewhere, disease-free interval (≤ 24 months versus > 24 months) or age at disease onset were not associated with metastatic disease survival.

When assessed separately by different biological subtypes, initial lymph node metastases predicted worse prognosis only in the patients with the luminal A subtype in univariate analysis (p = 0.019), but the small sample size of each subgroup limited the reliability of this analysis (data not shown).

In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis predicted poorer survival overall (HR, 2.17; 95% CI, 1.09-4.31; p = 0.027) when tumor size (T1 versus T2-4) (HR, 1.33; 95% CI, 0.71-2.47; p = 0.37) and ER status (negative versus positive) (HR 2.16; 95% CI 1.14-4.10; p = 0.018) were included in the analysis. The proportional hazards assumption was met in the analysis. Breast cancer subtype, Ki-67 expression or grade did not remain significant prognostic factors after multivariate analysis.

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

involvement at time of initial diagnosis was associated with shorter survival [12]. Another retrospective single-institute study also concluded that lymph node involvement at primary diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish retrospective registry study suggested that the initial lymph node status should be part of the prognostic index in recurrent metastatic breast cancer [31]. In addition to considerable change in the oncological treatments of breast cancer during the last decades, also surgical techniques, especially axillary procedures have developed considerably. The current results from the prospective data with modern treatments thus support and confirm earlier results.

In our study, any metastasis found in local lymph nodes at the time of definitive surgery was associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median survival was prolonged from 19–33 months in patients without lymph node metastases at the time of initial diagnosis. Although lymph node metastases in general is associated with other factors of poor prognosis, our study suggested that this result was independent of tumor size and ER expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these issues continues [36].

It has to be emphasized that all our patients had recurrent breast cancer, and our material did not include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless, the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer, and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9, 37]. As an other limitation, we were unable to address the results separately in subgroups, for example according to biological subtypes, due to relatively low number of patients with metastatic breast cancer. On the other hand, our study was based on a prospective cohort from a university clinic, and the patients were treated with up-to-date surgical and oncological treatment modalities.

Our results confirmed that ER negativity in primary tumor was associated with short survival for metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12, 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER status is also a predictor of poor prognosis [38]. Most previous studies have divided metastatic breast cancers only to three subgroups: ER/PR-positive, HER2-positive and TNBC. We used the widely recognized ESMO guidelines for subtyping our cases. Although the number of patients in each subgroup were rather limited, the patients with slowly proliferating, ER-responsive luminal A-like breast cancers still had significantly prolonged survival in metastatic breast cancer compared to other subtypes. TNBC has the worst outcome of all subtypes in metastatic breast cancer, a finding which was mirrored in our study [39].

Predicting the course of metastatic breast cancer is of primary importance in clinical practice; however, its status as a highly heterogenous disease at both the intrapatient and interpatient levels makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer guidelines recommend starting treatment with chemotherapy or even with a combination chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic breast cancer, such as initial nodal status, could be confirmed, these patients should receive more aggressive first-line metastatic breast cancer therapy.

In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial lymph node status may be a prognostic factor for metastatic disease course. Future studies should also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute number of metastatic lymph nodes, which were not addressed in our material. More research is also clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or whether they are purely an indicator of aggressive disease.

Contributorship statement

All authors contributed to the study design and conception. AJu initiated the collection of the prospective dataset. PK, AJä and NR were responsible for assessing statistical analyses. PK was a major contributor in writing the manuscript. All authors provided comments on drafts of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors report no competing interests.

Funding

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Data sharing statement

Data are available upon reasonable request.

Figure legends

Figure 1. Flow chart of the study participants.

Figure 2. Associations between primary tumor properties and survival in metastatic breast cancer.

In multivariate analysis, only ER expression and initial nodal status remained as significant factors.

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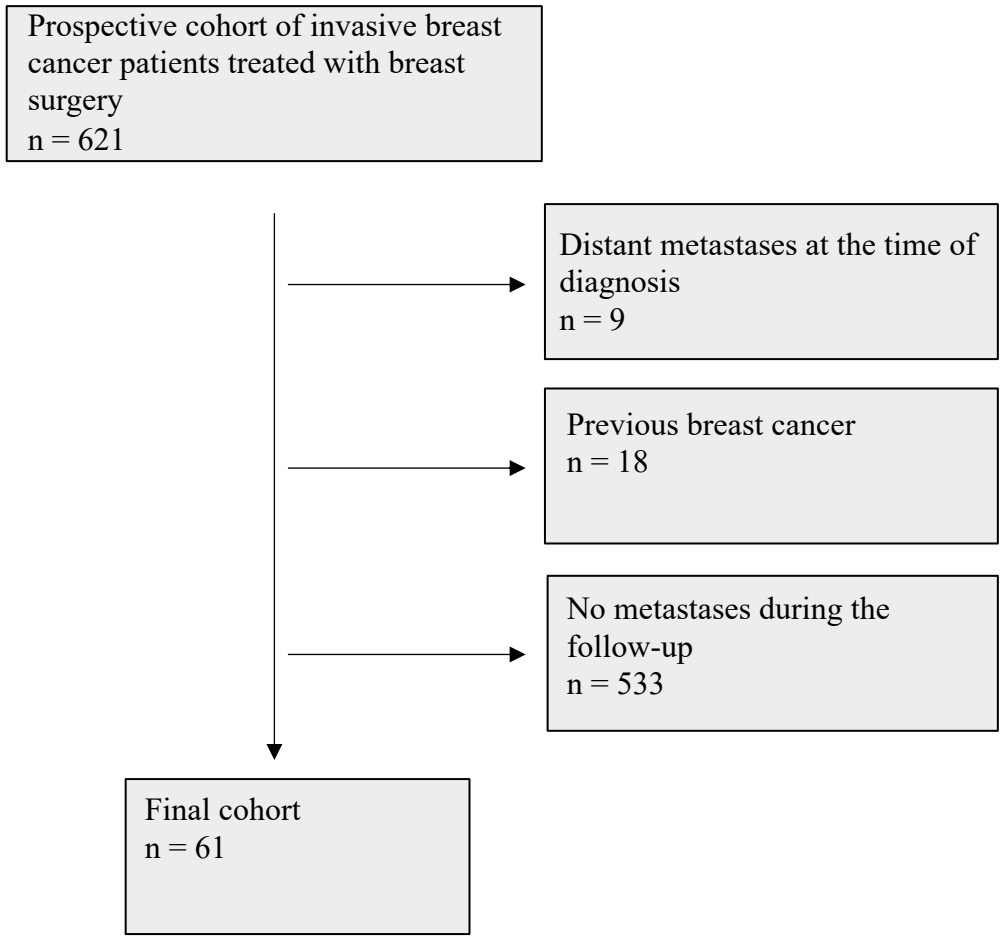
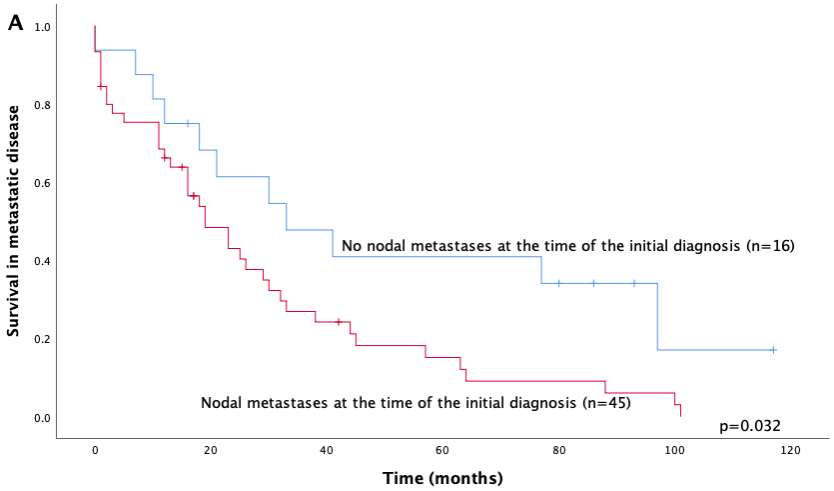
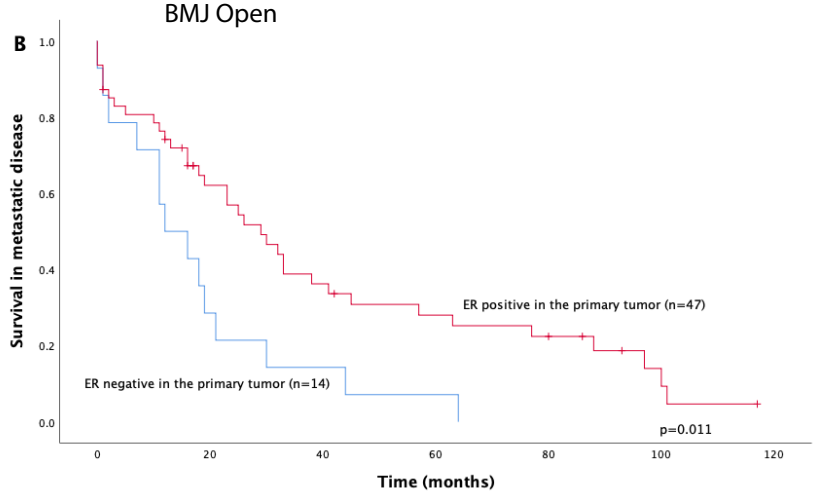
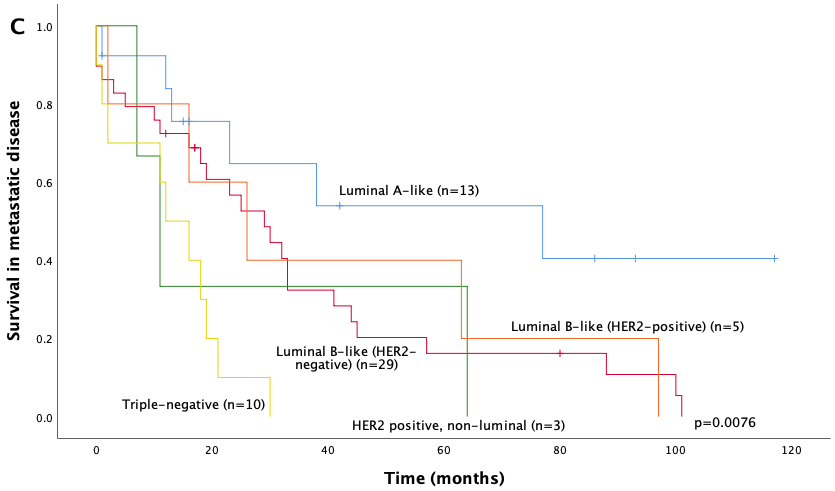
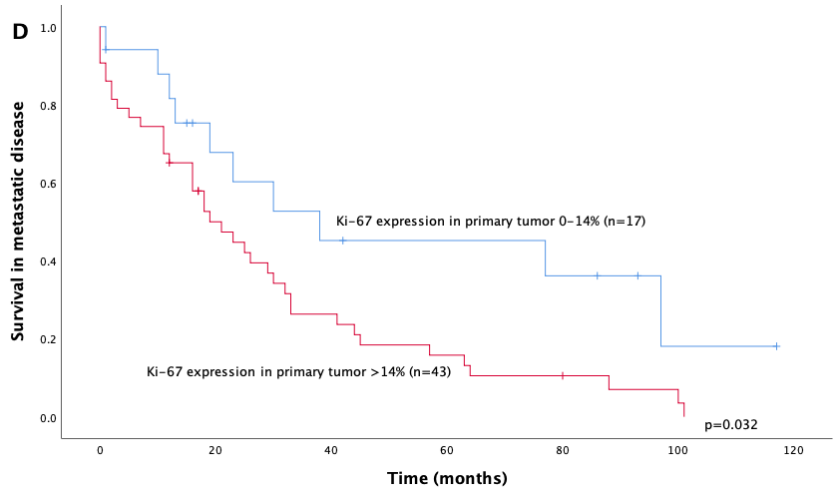
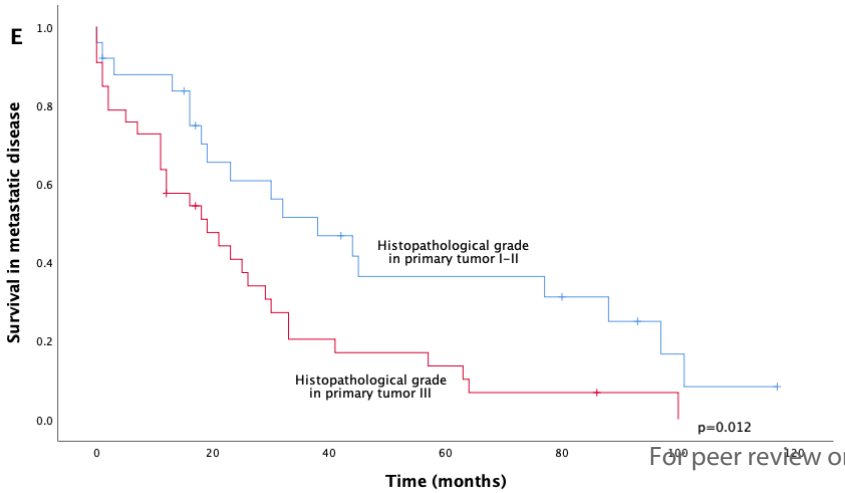

Prospective cohort of invasive breast cancer patients treated with breast surgery
n = 621
Distant metastases at the time of diagnosis
n = 9
Previous breast cancer
n = 18
No metastases during the follow-up
n = 533
Final cohort
n = 61











STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	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	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses		
Methods				
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3	
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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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	
Discussion			
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
Other information			
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.

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

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Keywords: estrogen receptor; metastatic breast cancer; metastasis; nodal status; prognosis

Word count: 2,274

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

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 the 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 the 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 ≥ 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 immunohistochemistry as previously described [16]. HER2 expression was studied using immunohistochemistry and chromogenic in situ hybridization (CISH) to confirm positive results. A positive result of six or more gene copies in CISH was considered HER2-positive [17] .

Ethical considerations

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

Statistical analyses

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

Table 1. Primary tumor characteristics.

	N (%)

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

PR expression	
Negative (0 %)	22 (36.1%)
Weak (1-9 %)	5 (8.2%)
Moderate (10-59 %)	5 (8.2%)
High (>59 %)	29 (47.5%)
HER2 status	
HER2-negative	52 (85.2%)
HER2-positive (CISH)	9 (14.8%)
Ki-67 expression	
Negative (< 5 %)	2 (3.3%)
Weak (5-14 %)	15 (24.6%)
Moderate (15-30%)	20 (32.8%)
High (> 30 %)	24 (39.3%)
Focality	
Unifocal	50 (82.1%)
Multifocal	11 (18.0%)
Subtype	
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%)
The first site of the distant metastasis	

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 lymph node-positive patients, it was 19.0 months. Only N0 versus N1-3 classification was significant. No prognostic differences between the patients with N1, N2 or N3 disease subtypes (p = 0.78) were detected.

Of the more traditional prognostic factors related to metastatic disease, ER positivity of the primary tumor (p = 0.011), Ki-67 expression ranging from 0%–14% (versus over 14%) in primary tumors (p = 0.032) and grade I–II (versus grade III) primary tumors (p = 0.012) were associated with better survival in metastatic disease in univariate analysis. Breast cancer subtype (determined from the initial surgical samples) also predicted survival with metastatic breast cancer (p = 0.0078), Also, the patients with luminal A-like breast cancer had significantly prolonged survival, when compared to all other subtypes (p = 0.017). Primary tumor size, PR or HER2 expression, the site of the first metastasis in bone versus elsewhere, disease-free interval (≤ 24 months versus > 24 months) or age at disease onset were not associated with metastatic disease survival.

When assessed separately by different biological subtypes, initial lymph node metastases predicted worse prognosis only in the patients with the luminal A subtype in univariate analysis (p = 0.019), but the small sample size of each subgroup limited the reliability of this analysis (data not shown).

In multivariate analysis, the presence of lymph node metastases at the time of initial diagnosis predicted poorer survival overall (HR, 2.17; 95% CI, 1.09-4.31; p = 0.027) when tumor size (T1 versus T2-4) (HR, 1.33; 95% CI, 0.71-2.47; p = 0.37) and ER status (negative versus positive) (HR 2.16; 95% CI 1.14-4.10; p = 0.018) were included in the analysis. The proportional hazards

assumption was met in the analysis. Breast cancer subtype, Ki-67 expression or grade did not remain significant prognostic factors after multivariate analysis.

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 involvement at time of initial diagnosis was associated with shorter survival [12]. Another retrospective single-institute study also concluded that lymph node involvement at primary diagnosis predicted unfavorable outcomes in metastatic breast cancer, although the first patients were enrolled in the study cohort in the 1960s [30]. In line with these studies, a Spanish retrospective registry study suggested that the initial lymph node status should be part of the prognostic index in recurrent metastatic breast cancer [31]. In addition to considerable change in the oncological treatments of breast cancer during the last decades, also surgical techniques, especially axillary procedures have developed considerably. The current results from the prospective data with modern treatments thus support and confirm earlier results.

In our study, any metastasis found in local lymph nodes at the time of definitive surgery was associated with dismal metastatic cancer survival outcomes. The Kaplan-Meier estimate for median survival was prolonged from 19–33 months in patients without lymph node metastases at the time of initial diagnosis. Although lymph node metastases in general is associated with other factors of poor prognosis, our study suggested that this result was independent of tumor size and ER expression. Node positivity may reflect not only higher metastatic potential of breast cancer, but it can possibly decipher impaired immunological microenvironments [32]. Interestingly, a recent paper by Ullah et al. using evolutionary genomic analyses of primary tumors and metastatic lesions suggested that ipsilateral axillary lymph node status in primary breast cancer was very useful for predicting the tumorigenic capability of the primary tumor; however, it did not drive metastasis *per se* [33]. Several other papers have suggested that metastatic lymph nodes did not eventually metastasize [34, 35]. However, it was also recently shown that the removal of metastatic axillary

lymph nodes resulted in the disappearance of circulating tumor DNA, and discussion on these issues continues [36].

It has to be emphasized that all our patients had recurrent breast cancer, and our material did not include samples from patients with *de novo* metastatic breast cancer. Whereas this makes the material more uniform, the results may not be suitable for generalizing to *de novo* metastatic breast cancers, which have a different natural course from recurrent breast cancers [10, 14]. Nevertheless, the prognostic value of ER status has also been previously demonstrated in recurrent breast cancer, and the initial lymph node status obviously cannot be evaluated in *de novo* metastatic cancers [8, 9, 37]. As an other limitation, we were unable to address the results separately in subgroups, for example according to biological subtypes, due to relatively low number of patients with metastatic breast cancer. On the other hand, our study was based on a prospective cohort from a university clinic, and the patients were treated with up-to-date surgical and oncological treatment modalities.

Our results confirmed that ER negativity in primary tumor was associated with short survival for metastatic disease. This obviously is not only due to the more aggressive nature of the cancer but also because the lack of ER-targeted treatments. Compelling evidence has demonstrated ER negativity in the primary tumor as an adverse prognostic factor in various previous studies [8, 9, 12, 14, 37]. ER status frequently changes in metastatic breast cancer, and the negative conversion of ER status is also a predictor of poor prognosis [38]. Most previous studies have divided metastatic breast cancers only to three subgroups: ER/PR-positive, HER2-positive and TNBC. We used the widely recognized ESMO guidelines for subtyping our cases. Although the number of patients in each subgroup were rather limited, the patients with slowly proliferating, ER-responsive luminal A-like breast cancers still had significantly prolonged survival in metastatic breast cancer compared to

other subtypes. TNBC has the worst outcome of all subtypes in metastatic breast cancer, a finding which was mirrored in our study [39].

Predicting the course of metastatic breast cancer is of primary importance in clinical practice; however, its status as a highly heterogenous disease at both the intrapatient and interpatient levels makes metastatic breast cancer very unpredictable [33, 40]. Current metastatic breast cancer guidelines recommend starting treatment with chemotherapy or even with a combination chemotherapy instead of hormonal treatments in patients with visceral crisis or rapidly progressing ER-positive, HER2-negative breast cancer [2, 41]. If novel adverse prognostic factors of metastatic breast cancer, such as initial nodal status, could be confirmed, these patients should receive more aggressive first-line metastatic breast cancer therapy.

In conclusion, our results strengthen the role of primary tumor ER negativity as an adverse prognostic factor in patients with recurrent breast cancer; however, they also suggest that initial lymph node status may be a prognostic factor for metastatic disease course. Future studies should also evaluate the prognostic power of isolated tumor cells, micrometastases and the absolute number of metastatic lymph nodes, which were not addressed in our material. More research is also clearly needed to clarify whether axillary lymph node metastases are able to seed metastatic cells or whether they are purely an indicator of aggressive disease.

Contributorship statement

All authors contributed to the study design and conception. AJu initiated the collection of the prospective dataset. PK, AJä and NR were responsible for assessing statistical analyses. PK was a

major contributor in writing the manuscript. All authors provided comments on drafts of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors report no competing interests.

Funding

There are no funders to report for this submission.

Data sharing statement

Data are available upon reasonable request.

Figure legends

Figure 1. Flow chart of the study participants.

Figure 2. Associations between primary tumor properties and survival in metastatic breast cancer. In multivariate analysis, only ER expression and initial nodal status remained as significant factors.

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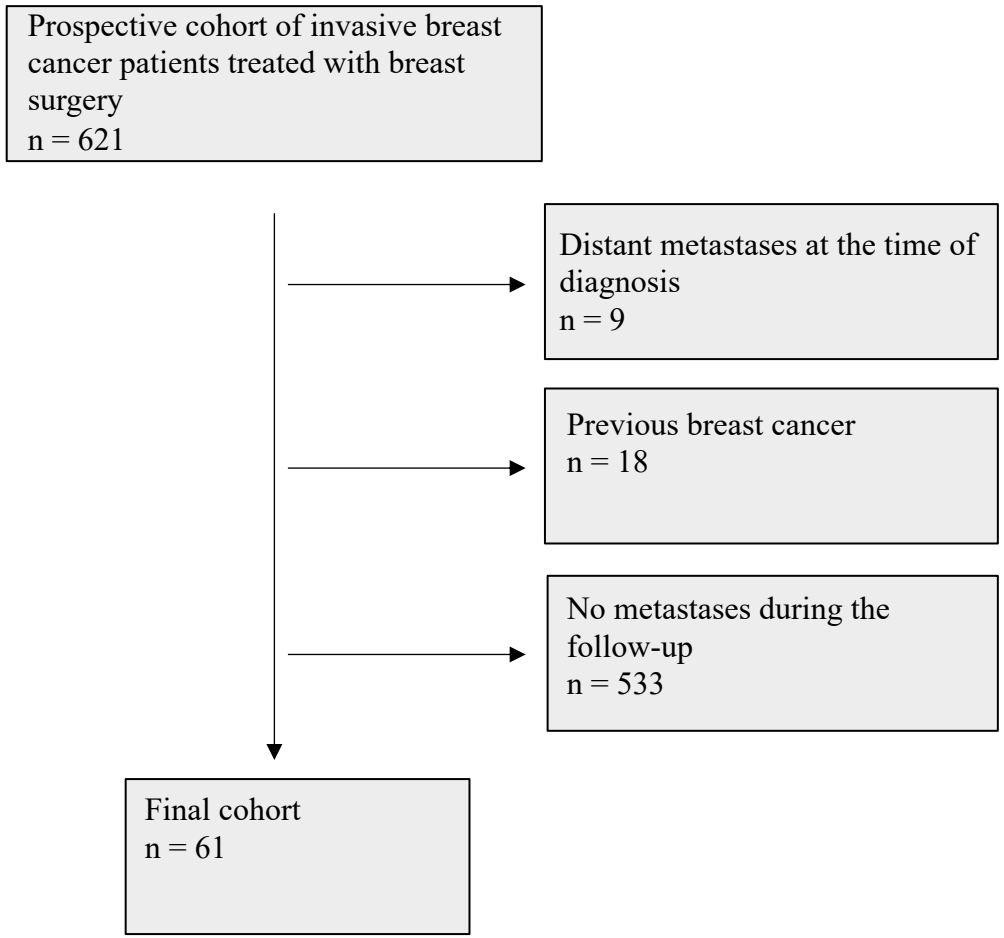
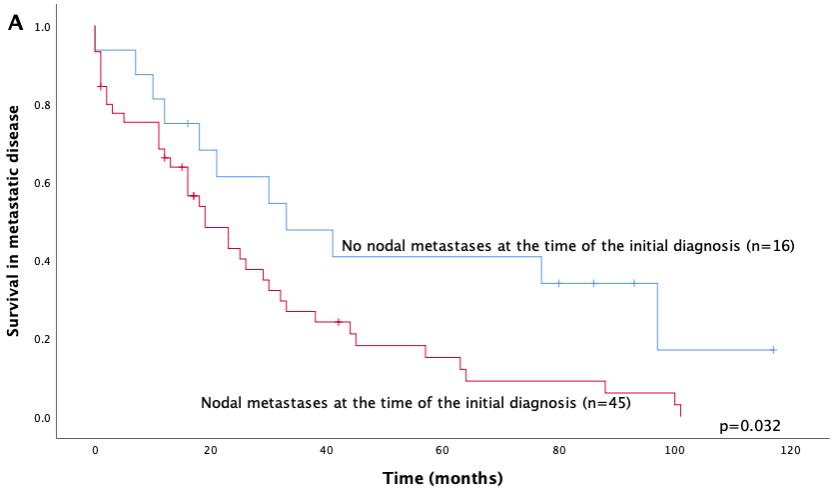
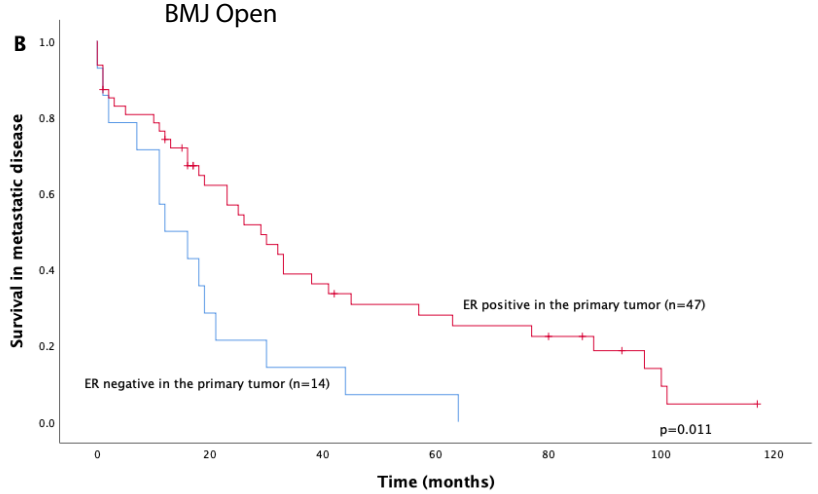
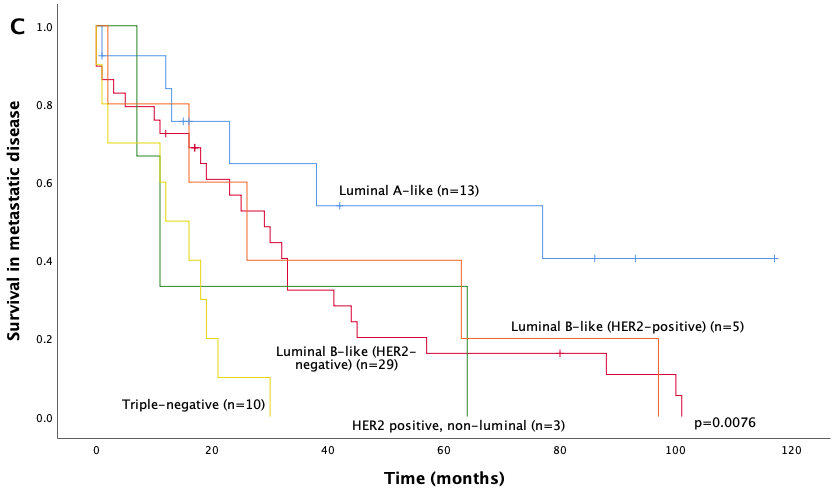
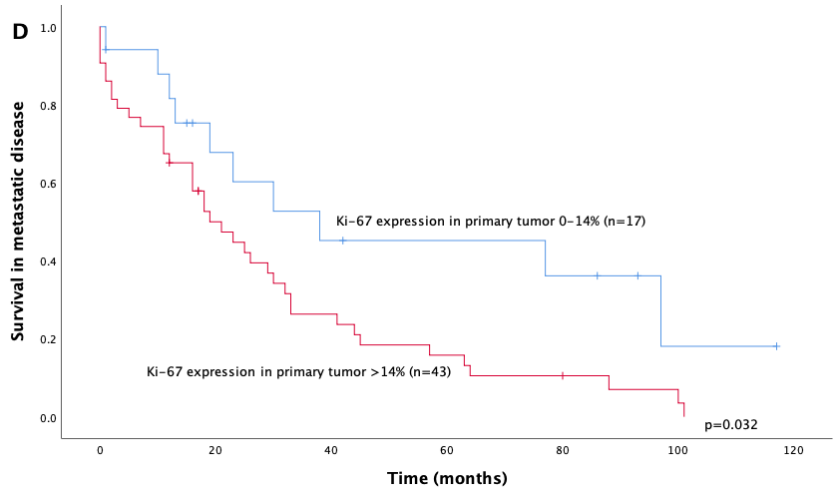
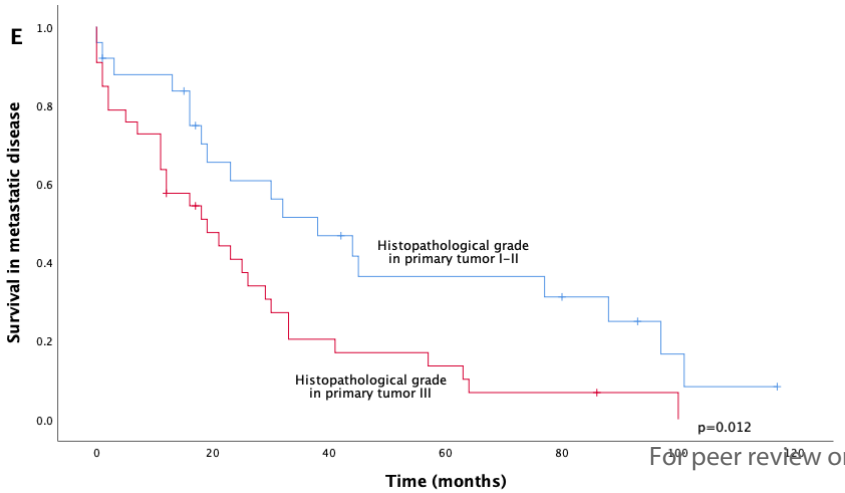

Prospective cohort of invasive breast cancer patients treated with breast surgery
n = 621
Distant metastases at the time of diagnosis
n = 9
Previous breast cancer
n = 18
No metastases during the follow-up
n = 533
Final cohort
n = 61











STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	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	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses		
Methods				
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*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—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 *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3	
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*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
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) *Cohort study*—Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	8
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—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	
Discussion			
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
Other information			
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.