The Breast 32 (2017) 79–86

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

Original article

Long-term outcome of breast cancer patients with pathologic N3a lymph node stage

Antonino Grassadonia [a, *], Patrizia Vici [b], Teresa Gamucci [c], Luca Moscetti [d], Laura Pizzuti [b], Lucia Mentuccia [c], Laura Iezzi [e, f], Maria Teresa Scognamiglio [e, f], Marinella Zilli [e, f], Jamara Giampietro [e, f], Vincenzo Graziano [e, f], Clara Natoli [a], Nicola Tinari [a]

[a] Department of Medical, Oral and Biotechnological Sciences, Center of Aging Sciences and Translational Medicine (CeSI-MeT), G. D'Annunzio University, Chieti, Italy
[b] Division of Medical Oncology 2, Regina Elena National Cancer Institute, Rome, Italy
[c] Medical Oncology Unit, ASL Frosinone, Frosinone, Italy
[d] Department of Oncology, Division of Medical Oncology, Belcolle Hospital, ASL Viterbo, Viterbo, Italy
[e] Medical Oncology Unit, SS Annunziata Hospital, Chieti, Italy
[f] Breast Medical Oncology Unit, G. Bernabeo Hospital, Ortona, Italy

ARTICLE INFO

Article history:
Received 30 October 2016
Received in revised form
22 December 2016
Accepted 25 December 2016

Keywords:
Breast cancer
Stage pN3a
Prognosis
Progesterone Receptor
Ki-67

ABSTRACT

Purpose: To evaluate factors influencing the long-term outcome of patients presenting with 10 or more metastatic axillary lymph nodes (pN3a) after surgery for primary breast cancer.
Method: Between January 1990 and December 2015, a total of 130 patients with pN3a breast cancer at surgery were identified in our Institutions and included in the study. Twenty-nine of them (22.3%) received neoadjuvant chemotherapy. The Multivariate Cox proportional hazards model was used to determine independent prognostic factors associated with DFS and OS.
Results: After a median follow-up of 6.4 years (range 0.87–25 years), 2 patients had a local relapse, 59 distant metastases (1 with local relapse) and 52 patients died. The 5-year DFS and OS rates were 61.8% and 71.5%, respectively. At multivariate analysis, pN3a stage after neoadjuvant chemotherapy (ypN3a) was significantly associated with increased risk of recurrence (HR 1.92, $p = 0.02$) and death (HR 2.05, $p = 0.029$). Absence of progesterone receptor (PR) expression was the most important tumor characteristic associated with poor prognosis, both in terms of recurrence (HR 2.55, $p < 0.001$) and death (HR 2.23, $p = 0.019$). High levels of Ki-67 index (≥20%) were significantly associated with a shorter OS (HR 2.03, $p = 0.027$), but not with DFS.
Conclusions: The results of this study indicate that ypN3a stage, lack of expression of PR, and Ki-67 ≥ 20% negatively affect long-term outcome of patients with pN3a breast cancer.

1. Introduction

Recent advances in the understanding of molecular biology of breast cancer have led to the identification of specific molecular subtypes that require different therapeutic approaches and show different prognosis [1,2]. However, the degree of regional axillary lymph node involvement remains one of the most important factors to be considered for long-term outcome [3].

Data from the National Cancer Data Base showed that almost half of the patients diagnosed in 2001–2002 with metastases in 10 or more axillary lymph nodes, classified as stage IIIC, died because of breast cancer within 5 years from surgery, independently from the size of primary tumor [3]. Fortunately, at least in developed countries, the availability of breast cancer screening and the increased awareness of women regarding prevention have reduced the incidence of stage III breast cancer to 10–15% of all newly diagnosed cases [4].

Abbreviations: CI, confidential interval; CISH, chromogenic in situ hybridization; DFS, disease free survival; ER, estrogen receptor; FISH, fluorescence in situ hybridization; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IHC, immunohistochemistry; LNR, lymph node ratio; LVI, lymphovascular invasion; OS, overall survival; PR, progesterone receptor.

* Corresponding author.
E-mail address: grassadonia@unich.it (A. Grassadonia).

http://dx.doi.org/10.1016/j.breast.2016.12.018
0960-9776/

Although improved survival rates have been observed with modern therapies, including (i) chemotherapy regimens containing anthracyclines and taxanes, (ii) aromatase inhibitors for hormone receptor-positive tumors, and (iii) trastuzumab for Human Epidermal Growth Factor Receptor 2 (HER2)-positive tumors [5], patients with stage III breast cancer maintain a dismal prognosis compared to those at earlier stages. However, the great heterogeneity of breast cancer in terms of molecular expression and biological behavior largely accounts for different rates of disease relapse and progression even among patients with pN3a tumors [6,7]. In order to identify possible clinical and/or pathological factors that could influence the long-term outcomes of patients at this stage of disease, we carried out a retrospective study in a cohort of patients consecutively treated in five Italian Cancer Institutions.

2. Subjects and methods

2.1. Patients

The study population was identified by selecting patients registered as having pN3a breast cancer between January 1990 and December 2015 in five Italian Cancer Centers. In all cases, mastectomy or breast-conserving surgery, along with axillary lymph node dissection, were performed with curative intent. Patients diagnosed with distant metastases within 6 months from surgery were excluded.

The following clinical characteristics were collected and included in the analysis: menopausal status, type of surgery, pathological T stage, number of metastatic lymph nodes, lymph node ratio (LNR) with a cut-off set at 60%, and type of therapy. This latter aspect included information on the neoadjuvant or adjuvant setting, the use of anthracyclines and/or taxanes and the use of trastuzumab in patients with HER2-positive tumor.

According to our institution recommendations all patients were candidate to adjuvant radiotherapy, but a few were not treated because unfit for age or comorbidities.

2.2. Pathological assessment

Surgical specimens were processed to determine the expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor type 2 receptor (HER2) and Ki-67 by immunohistochemistry (IHC). Cut-off for positivity of ER and PR expression was chosen at 10% of tumor cells stained. HER2 status was assessed by HercepTest (Dako Italia, Milan Italy) and scored as follows: score 0, membrane staining in <10% of tumor cells; score 1+, partial and/or faint membrane staining in >10% of tumor cells; score 2+, weak to moderate, complete membrane staining in >10% tumor cells and score 3+, strong, complete membrane staining in >10% of tumor cells. Fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) was carried out on all tumors with HercepTest 2+. Tumors with a score 3 + by IHC or gene amplification by FISH or CISH were considered as HER2-positive. IHC detection of Ki-67 was performed using the MIB-1 antibody, and a cut-off of 20% was used.

Molecular subtypes were classified as “luminal A”: ER-positive, PR $\geq$ 20%, Ki-67 < 20%, HER2-negative; “luminal B/HER2-negative”: ER and/or PR-positive, Ki-67 $\geq$ 20% and/or PR <20%, HER2-negative; “luminal B/HER2-positive”: ER and/or PR-positive, any Ki-67, HER2-positive; “HER2 enriched”: ER and PR-negative, HER2-positive; “triple negative”: ER and PR-negative, HER2-negative.

Lymphovascular invasion (LVI) was evaluated by IHC using D2-40 and CD31 monoclonal antibodies. Nuclear grade was assessed according to the Nottingham grading system.

Most of IHC assessments were centrally re-evaluated to minimize variations in the determination of tumor molecular characteristics.

2.3. Statistical analysis

The Kaplan-Meier method was used to calculate the 5-year rates of disease free survival (DFS) and overall survival (OS). The Log-rank test and the Cox proportional hazards model were used to compare survival curves and independent prognostic factors, respectively.

Multivariate analyses were performed using the Cox proportional hazards model to determine independent prognostic factors with significant impact on DFS and OS. Covariates included in the Cox model were all significant predictors at univariate analysis. A p value of 0.05 or less was considered as statistically significant. All statistical analyses were performed using SPSS® software 11.0 (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Patient and tumor characteristics

One hundred and thirty patients with pN3a breast cancer were identified and included in this study. Patient and tumor characteristics are illustrated in Tables 1 and 2. The median age at surgery was 58 years (range 29–85 years). Most patients (76.2%) were postmenopausal. Mastectomy or breast-conserving surgery was performed in 69 (53.1%) and 61 (46.9%) patients, respectively. Pathological examination showed small tumors (<2 cm) in only 30 (23.1%) patients and revealed more than 20 metastatic axillary lymph nodes in 73 (56.2%) patients. LNR, i.e. the ratio between positive lymph nodes and the total removed lymph nodes, was more than 60% in 106 (81.5%) patients.

In 29 (22.3%) cases breast surgery was preceded by neoadjuvant chemotherapy. None of these patients received further adjuvant chemotherapy after surgery. Among patients who underwent surgery immediately after diagnosis, 88 (67.7%) were treated with adjuvant chemotherapy and 13 (10%) received only endocrine therapy. The most frequently used chemotherapy regimens included anthracyclines and/or taxanes (79.2%). All patients with hormone receptor-positive tumor, initially treated with adjuvant/neoadjuvant chemotherapy, received also endocrine therapy afterward. Trastuzumab for 1 year was given in 20 (46.5%) out of 43 patients with HER2-positive disease. All but four patients received adjuvant radiotherapy.

Invasive ductal carcinoma and G2/G3 grade were the most frequent tumor characteristics, 66.9% and 83.9%, respectively. Hormone receptor positivity was observed in 90 (69.2%) cases, HER2 positivity in 43 (33.1%), and high Ki67 ($\geq$20%) in 52 (40.0%). According to the molecular subtype definition, tumor was classified as luminal A in 26 (20.0%), luminal B/HER2-in 38 (29.2%), luminal B/HER2+ in 26 (20.0%), HER2 enriched in 15 (11.5%), and triple negative in 17 (13.1%) patients. LVI was observed in 53 (40.7%) cases.

3.2. Long-term outcome

Median follow-up was 6.4 years (range 0.87–25 years) for all patients. During the follow-up, 2 patients had local relapse, 59 patients had distant metastases (1 patient both local relapse and distant metastases) and 52 patients died, including 4 from causes not related to cancer. In the overall population, median DFS and OS were 8.46 years and 11.83 years, respectively (Fig. 1a and b). The estimated 5-year DFS and OS rates were 61.8% and 71.5%, respectively (Fig. 1a and b). Among patients who recurred, the median

Table 1
Patient characteristics.

Variable	n (%)
Median age at surgery (years): 58 (range 29–85)	
Menopausal status, n(%)	
Premenopausal	31 (23.8)
Postmenopausal	99 (76.2)
Type of surgery, n(%)	
Breast Conservative Surgery	61 (46.9)
Mastectomy	69 (53.1)
Pathological T stage, n(%)	
T1	30 (23.1)
T2	72 (55.4)
T3	22 (16.9)
T4	6 (4.6)
Number of metastatic lymph nodes, n(%)	
10-19	57 (43.8)
≥ 20	73 (56.2)
Lymph node ratio (LNR), n(%)	
≤ 60%	24 (18.5)
> 60%	106 (81.5)
Type of therapy, n(%)	
Neoadjuvant chemotherapy	29 (22.3)
Adjuvant chemotherapy	88 (67.7)
Adjuvant endocrine therapy only	13 (10.0)
Chemotherapy regimen, n(%)	
Anthracyclines and/or Taxanes	103 (79.2)
Others	14 (10.8)
Trastuzumab in HER2-positive, n(%)	
Yes	20 (15.4)
No	23 (17.7)

time between surgery and recurrence was 2.7 years (range, 0.56–10.93 years). The median time from surgery to death was 3.71 years (range, 1.0–25.5 years).

Table 2
Tumor characteristics.

Variable	n (%)
Histologic type, n(%)	
Ductal	87 (66.9)
Lobular	23 (17.7)
Ductal-lobular	17 (13.1)
Others	3 (2.3)
Grade, n(%)	
G1	18 (13.8)
G2	60 (46.2)
G3	49 (37.7)
Unknown	3 (2.3)
Estrogen Receptor (ER), n(%)	
< 10%	38 (29.2)
≥ 10%	92 (70.8)
Progesteron Receptor (PR), n(%)	
< 10%	64 (49.2)
≥ 10%	66 (50.8)
HER2, n(%)	
Positive	43 (33.1)
Negative	81 (62.3)
Unknown	6 (4.6)
Ki-67, n(%)	
< 20%	65 (50.0)
≥ 20%	52 (40.0)
Unknown	13 (10.0)
Molecular subtype, n(%)	
Luminal A	26 (20.0)
Luminal B/HER2-	38 (29.2)
Luminal B/HER2+	26 (20.0)
HER2 enriched	15 (11.5)
Triple Negative	17 (13.1)
Lymphovascular invasion, n(%)	
No	17 (13.1)
Yes	53 (40.7)
Unknown	60 (46.2)

3.3. Prognostic factors

Factors associated with DFS and OS are shown in Table 3. At Kaplan-Mayer analysis, the persistence of disease in 10 or more axillary lymph nodes after neoadjuvant chemotherapy (ypN3a) was found to be predictive of shorter DFS and OS (Fig. 2a and b). After 5 years of follow-up, the estimated cumulative DFS rate was 46.3% for patients treated with neoadjuvant chemotherapy and 66.5% for patients treated with surgery followed by adjuvant therapy (HR 2.11; 95% CI, 1.31–4.92; p = 0.006). The estimated cumulative OS rate in the two groups was 50.4% and 77.5% (HR 1.94; 95% CI, 1.10–4.73; p = 0.026), respectively. Among patients who received adjuvant therapy, those treated with chemotherapy had a better prognosis compared to those treated with endocrine therapy alone, with a significant longer DFS (HR 0.4; 95% CI, 0.09–0.77; p = 0.015) (Fig. 2a), and a trend for OS (HR 0.45; 95% CI 0.09–1.05; p = 0.06) (Fig. 2b). Patients with HER2-positive tumor who received trastuzumab showed also a trend towards improved survival, although the difference was not statistically significant (data not showed).

Clinico-pathological characteristics such as menopausal status, type of surgery, tumor size, tumor grade, HER2-positivity, number of metastatic lymph nodes, LNR, and LVI were not associated with prognosis.

When comparing the survival curves of patients with different tumor subtypes (Fig. 3a and b), those with hormone receptor positive tumors, in particular luminal A, showed a better prognosis. When luminal A was used as reference variable in the Kaplan-Mayer analysis, these patients showed a significant lower risk of recurrence and death compared to those with non-luminal subtypes. In particular, the 5-year DFS rates were 87.6% for luminal A tumor, 46.9% for HER2-enriched (HR 2.63, 95% CI, 1.01–7.63; p = 0.047), and 43.8% for triple negative (HR 3.03; 95% CI, 1.13–10.9; p = 0.044); the 5-year OS rates were 86.6%, 61.4% (HR 2.63, 95% CI, 0.81–9.01; p = 0.050) and 37.3%, (HR 4.17; 95% CI, 1.67–17.2; p = 0.005) in each subtype, respectively.

The expression of PR rather than ER appeared to significantly influence prognosis. In fact, compared to patients with PR-positive tumor, those with PR-negative tumor had a higher risk of recurrence (HR 2.6, 95% CI 1.47–4.45; p < 0.001) and death (HR 2.40, 95% CI 1.39–4.17; p = 0.002) (Fig. 4a and b).

Tumor cell proliferation, as evaluated by Ki-67 expression, was another factor associated with long-term outcome. A Ki-67 index ≥20 was predictive of worse OS (HR 2.00; 95% CI, 1.09–3.77; p = 0.025), but not DFS (Fig. 5a and b).

At multivariate analysis (Table 4), PR negativity and ypN3a stage demonstrated to be independent factors associated with poor DFS (HR 2.55; 95% CI, 1.48–4.38; p < 0.001 and HR 1.92; CI, 1.11–3.32; p = 0.020, respectively) and OS (HR 2.23; 95% CI, 1.14–4.36; p = 0.019 and HR 2.05; 95% CI, 1.08–3.92; p = 0.029, respectively). Ki-67 ≥ 20 was also an independent variable of poor OS (HR 2.03; 95% CI, 1.09–3.80; p = 0.027).

4. Discussion

In this study we evaluated the clinical outcomes in a cohort of patients with confirmed pN3a stage breast cancer and investigated the relationships between clinicopathological characteristics and prognosis.

In the overall population, the estimated 5-year DFS and OS rates were 61.8% and 71.5%, respectively, more favorable than published historical data (about 40% and 55%, respectively) [6–8] and coherent with more recent studies using modern standard treatments (46.2% and 69.8%, respectively) [9,10]. In fact, in the present study, patients who received chemotherapy were mostly treated with anthracyclines and/or taxanes regimens (88%) and half of

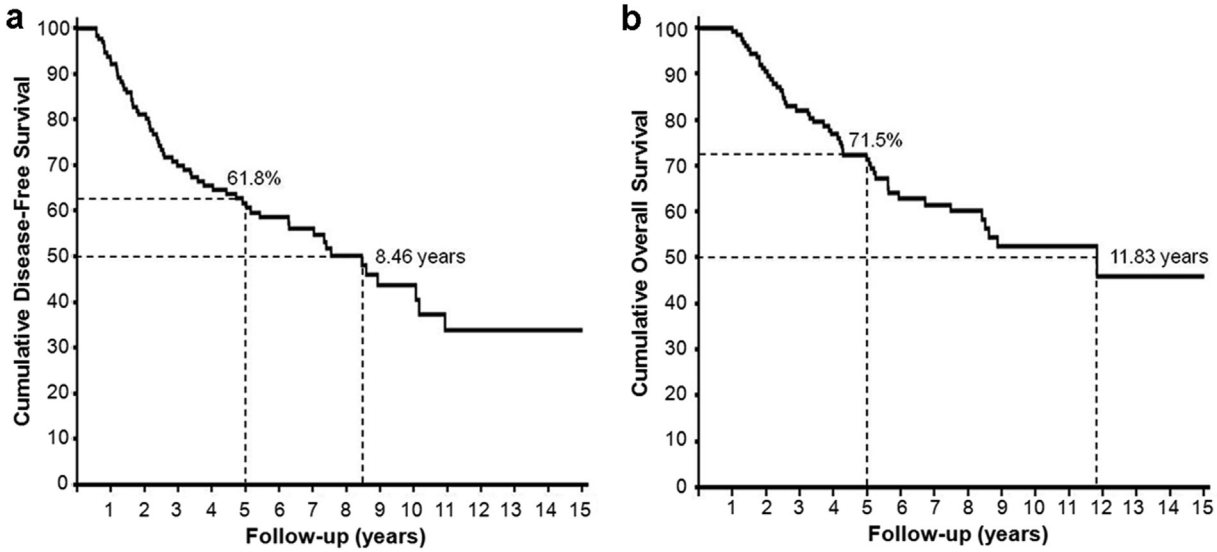

Fig. 1. Cumulative disease-free survival (a) and overall survival (b) of patients with pN3a breast cancer.

those with HER2-positive tumor received trastuzumab (all patients diagnosed after 2005, when trastuzumab was approved in Italy). Among HER2-positive tumor, patients treated with trastuzumab appeared to have a trend to better survival, but the benefit resulted not significant, likely due to the small number of patients.

We found that ypN3 stage, as expected, was predictive of poor prognosis. Lack of reaching a pathological complete response after neoadjuvant chemotherapy is a well established factor associated with higher risk of recurrence and death, especially in triple negative or HER2-positive breast cancer [11]. Absence of down-staging after neoadjuvant chemotherapy has been also reported as a strong predictive factor of poor outcome [12–14]. A residual bulky lymph nodal status is indicative of a biologically aggressive tumor resistant to neoadjuvant treatment and is negatively associated with survival [15,16]. Consistently, the results presented in this study confirm the very poor prognosis of patients with ypN3a disease in clinical practice, suggesting the need for adjuvant clinical trials in this subset of patients.

When tumor characteristics were analyzed, luminal A tumor was the molecular subtype associated with longer survival, indicating that in pN3a breast cancer a full endocrine-sensitive phenotype confers a better prognosis than one partially endocrine-sensitive (luminal B) or endocrine-resistant (HER2 enriched or triple negative). Several studies exploring long-term outcomes in early breast cancer have consistently showed that luminal A is by far the tumor subtype with the lowest relapse rate and the best prognosis [17–19]. It also differs from luminal B in terms of disease recurrence and sensitivity to chemotherapy [20–22]. In this study, to discriminate between luminal A and luminal B subtypes, we set the cut-off point of Ki-67 at <20% and that of PR at ≥20%, as suggested by recent pathological revisions aimed at reclassifying luminal A tumor [23–25].

In our population with pN3a disease, compared to patients with luminal A tumor, those with luminal B experienced similar outcomes, with a positive trend for luminal A, while patients with HER2 enriched and triple negative tumor showed a significant higher risk of recurrence (HR 2.63 and HR 3.03, respectively) and death (HR 2.63 and HR 4.17, respectively). It has been reported that luminal B tumor is more responsive to chemotherapy than luminal A [26–29], but it seems to retain the same endocrine sensitiveness [30–34]. To fully interpret the survival curves of the different molecular subtypes, it has to be considered that in our population almost all the patients (90%) received chemotherapy and all those with luminal tumor received endocrine therapy. Thus, chemotherapy would have balanced the better prognosis of luminal A compared to luminal B, and the subsequent endocrine therapy would have given a similar benefit to both luminal tumors.

Interestingly, in this study, factors related to tumor biological behavior, such as total number of metastatic lymph nodes, LNR, and LVI, did not correlate with prognosis, while intrinsic tumor cell characteristics, such as Ki-67 index and PR expression, were significantly and independently associated with long-term survival.

The number of metastatic lymph nodes and the lymph node ratio have been reported as prognostic factor in pN3a disease [8,35,36]. In particular, the greatest difference in survival has been found when a value of 0.60 was set as the cut-off point for LNR [36]. In the present study neither lymph node involvement nor LNR affected prognosis, presumptively because most of our patients (81.5%) were above LNR 0.60.

LVI has been reported as an additional prognostic factor in patients with breast cancer [37–39], including pN3a disease [8]. Unfortunately, in our cohort, data on LVI were not available in almost half of the patients and statistical analysis failed to find a significant association between LVI and prognosis.

Conversely, we found that patients with Ki-67 ≥ 20% had higher risk of death compared to those with Ki-67 < 20% (HR 2.00, $p = 0.025$). The lack of standard methods for Ki-67 determination and scoring has discouraged its routine use for the estimation of prognosis or adjuvant treatment decisions in patients with breast cancer [40]. However, in early breast cancer, high Ki-67 index has been reported to be associated with worse prognosis [41–43] and a cut-off of 14% has been proposed to separate luminal A (≤14%) from luminal B (>14%), an important decision level to recommend chemotherapy versus endocrine therapy [44,45]. Subsequent revision of luminal subtypes has raised Ki-67 cut-off to 20%, especially when PR expression is more than 20% [24,25]. So we used the Ki-67 cut off of 20% in the present study. On the best of our knowledge, this is the first evidence supporting a prognostic role of Ki-67 in pN3a breast cancer.

Overwhelming evidence supports a prognostic role of PR in breast cancer [46]. PR expression ≥20% has been proposed in the pathological definition of luminal A, even in presence of low Ki-67

Table 3
Univariate analysis.

Variable	n	DFS			OS		
		5-yr (%)[a]	HR (95% CI)	*p-value*	5-yr (%)[a]	HR (95% CI)	*p-value*
Menopausal state							
Pre	31	61.3	1.00		72.3	1.00	
Post	99	62.9	0.99 (0.55–1.74)	*0.962*	69.0	1.01 (0.53–1.88)	*0.989*
Type of surgery							
Breast Conservative Surgery	61	64.0	1.00		73.8	1.00	
Mastectomy	69	59.7	1.08 (0.66–1.81)	*0.729*	69.3	1.39 (0.80–2.50)	*0.233*
Pathological T stage							
T1	30	70.5	1.00		76.9	1.00	
T2	72	58.8	1.27 (0.66–2.38)	*0.494*	69.3	1.58 (0.74–3.12)	*0.255*
T3	22	62.4	1.30 (0.54–3.23)	*0.541*	78.5	1.79 (0.64–5.30)	*0.253*
T4	6	50.0	1.31 (0.39–4.76)	*0.629*	50.0	1.76 (0.48–13.1)	*0.273*
Metastatic lymph nodes							
10-19	57	58.3	1.00		67.6	1.00	
≥ 20	73	64.5	0.77 (0.48–1.33)	*0.383*	74.4	0.93 (0.53–1.63)	*0.788*
Lymph node ratio (LNR)							
≤ 60%	24	69.8	1.00		72.9	1.00	
> 60%	106	60.0	1.46 (0.75–2.63)	*0.293*	71.2	1.54 (0.73–2.94)	*0.283*
Neoadjuvant chemotherapy							
No	101	66.5	1.00		77.5	1.00	
Yes	29	46.3	2.11 (1.31–4.92)	***0.006***	50.4	1.94 (1.10–4.73)	***0.026***
Chemotherapy regimen							
Anthracyclines and/or Taxanes	103	63.7	1.00		73.9	1.00	
Others	14	56.7	1.05 (0.52–2.13)	*0.876*	61.8	0.92 (0.43–1.92)	*0.802*
Grade							
G1	18	59.6	1.00		88.9	1.00	
G2	60	53.2	1.10 (0.53–2.27)	*0.808*	63.5	1.56 (0.68–3.33)	*0.315*
G3	49	68.6	0.59 (0.38–1.92)	*0.696*	72.0	1.30 (0.54–3.10)	*0.560*
Lymphovascular invasion							
No	17	64.1	1.00		79.9	1.00	
Yes	53	47.5	1.67 (0.73–3.39)	*0.250*	65.5	2.04 (0.77–4.25)	*0.171*
Estrogen Receptor (ER)							
≥ 10%	92	64.9	1.00		77.3	1.00	
< 10%	38	54.2	1.23 (0.70–2.18)	*0.456*	57.4	1.47 (0.83–2.86)	*0.171*
Progesteron Receptor (PR)							
≥ 10%	64	80.6	1.00		86.0	1.00	
< 10%	66	44.1	2.61 (1.47–4.45)	***<0.001***	57.9	2.40 (1.39–4.17)	***0.002***
ER and PR > 50%							
Yes	43	84.5	1.00		86.4	1.00	
No	85	49.9	2.13 (1.18–3.33)	***0.010***	63.8	2.5 (1.27–4.00)	***0.006***
HER2							
Negative	81	62.1	1.00		72.9	1.00	
Positive	43	55.3	2.01 (0.68–2.00)	*0.579*	64.8	1.05 (0.57–1.92)	*0.871*
Ki-67							
< 20%	65	64.3	1.00		80.4	1.00	
≥ 20%	52	55.0	1.05 (0.61–1.81)	*0.857*	56.6	2.00 (1.09–3.77)	***0.025***
Molecular subtype							
Luminal A	26	87.6	1.00		86.6	1.00	
Luminal B/HER2-	38	55.7	2.15 (0.90–4.34)	*0.090*	77.7	1.15 (0.76–5.00)	*0.164*
Luminal B/HER2+	26	60.2	1.89 (0.72–4.65)	*0.199*	66.9	2.13 (0.70–5.74)	*0.196*
HER2 enriched	17	46.9	2.63 (1.01–7.63)	***0.047***	61.4	2.63 (0.81–9.01)	***0.044***
Triple Negative	15	43.8	3.03 (1.13–10.9)	***0.030***	37.3	4.17 (1.67–17.2)	***0.005***

In bold p-values statistically significant.
[a] Unadjusted Kaplan-Meier estimates.

(<14%) [23]. PR positivity has been reported to be a marker of long-term benefit from adjuvant tamoxifen in patients with ER-positive tumors [47]. Recently, it has been demonstrated that in cancer cells PR is not just a marker of functional ER, but is actively involved in the transcription process of unique ER-sensitive genes associated with good clinical outcome [48]. Consistently, in our pN3a population, we found that patients with PR-negative tumor had higher risk of recurrence (HR 2.61, $p < 0.001$) and death (HR 2.40, $p = 0.002$) compared to those with PR-positive tumor. Multivariate analyses confirmed PR positivity as a favorable prognostic factor independent from other clinicopathological variables.

As mentioned above, Ki-67 and PR have been extensively studied as prognostic factors in early stage breast cancer, but have been less investigated in more advanced stages. Interestingly, we showed that Ki-67 and PR maintain a prognostic role in locally advanced pN3a breast cancer. However, the interpretation of this result should be taken with caution because of possible biases linked to the retrospective nature of this study, especially when Ki-67 and PR are analyzed as single parameters extrapolated from the definition of molecular subtype.

In conclusion, despite the limitations due to the restricted sample size and the retrospective design, the present study provides real-world evidence on the prognosis of patients with pN3a breast cancer. It confirms the poor prognosis for patients with ypN3 residual tumor and corroborates the influence of molecular subtypes, along with tumor Ki-67 index and PR expression, on the long-term outcome of patients with locally advanced disease.

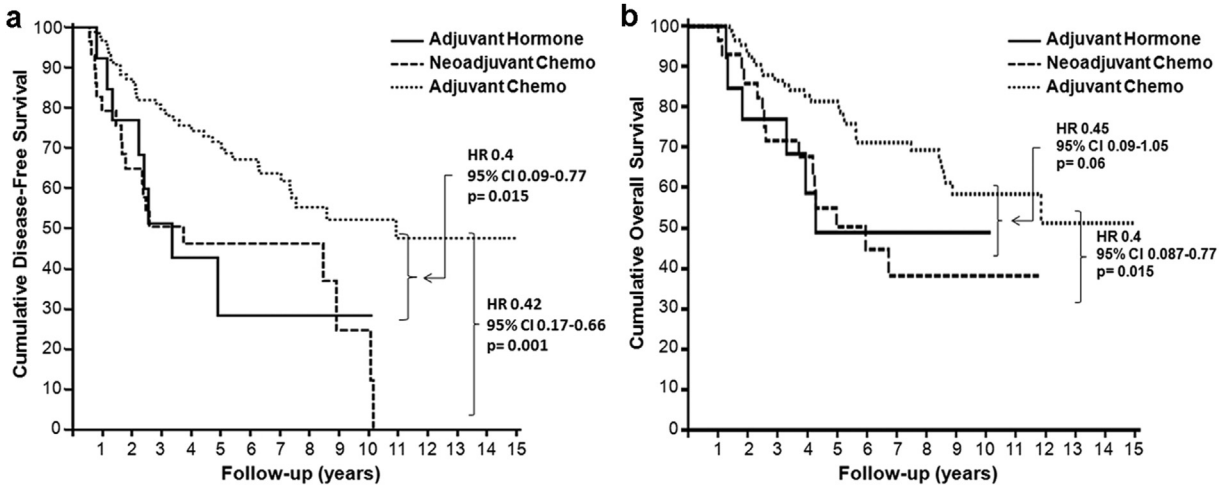

Fig. 2. Cumulative disease-free survival (a) and overall survival (b) stratified by type of initial treatment after diagnosis of breast cancer. All patients with hormone receptor-positive breast cancer received endocrine therapy after chemotherapy.

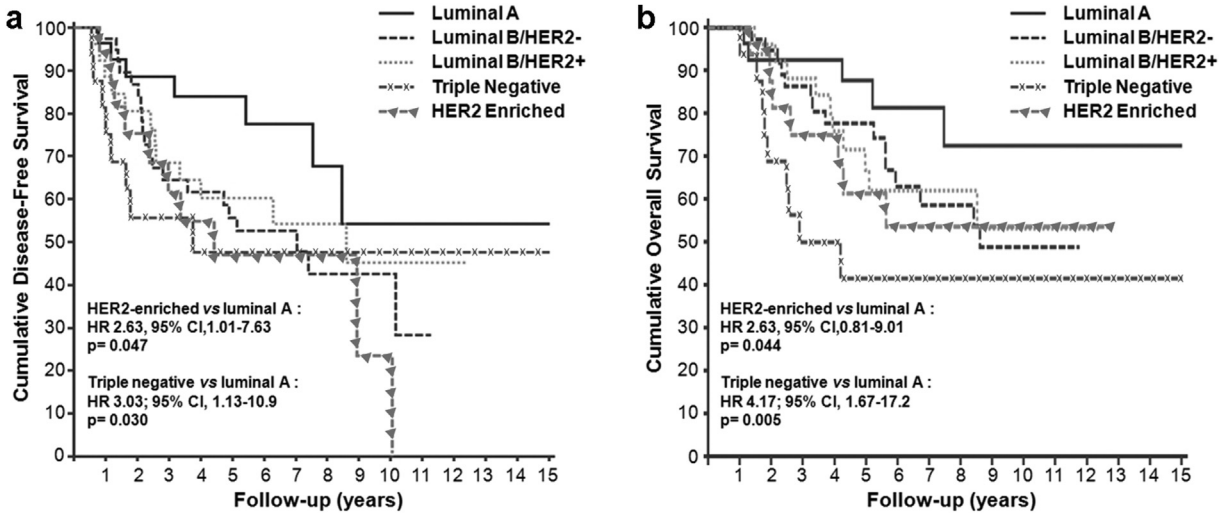

Fig. 3. Cumulative disease-free survival (a) and overall survival (b) stratified by molecular subtype.

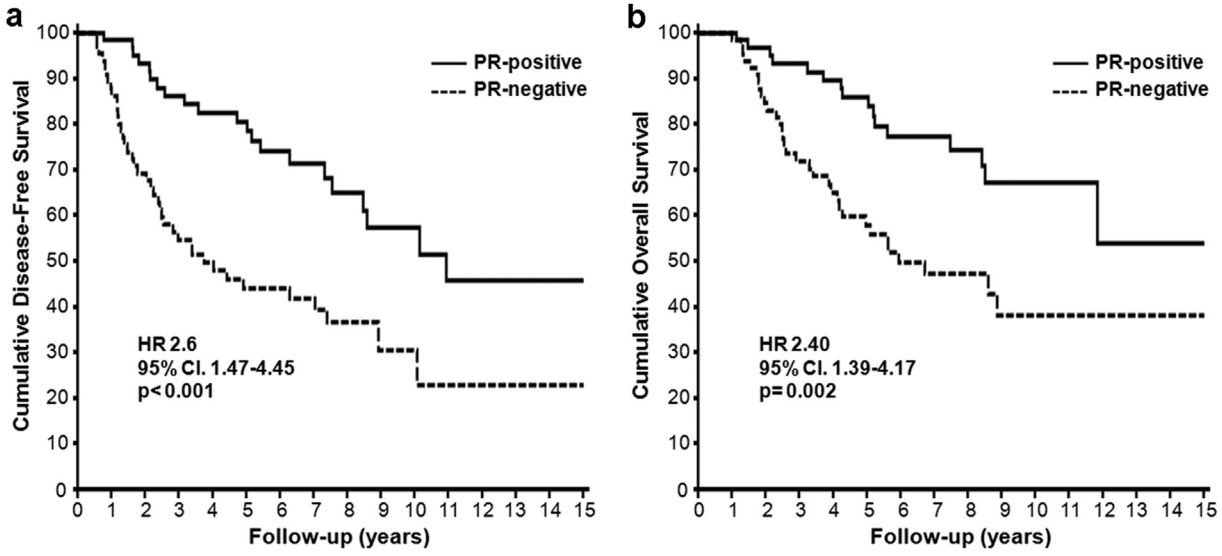

Fig. 4. Cumulative disease-free survival (a) and overall survival (b) stratified by PR status.

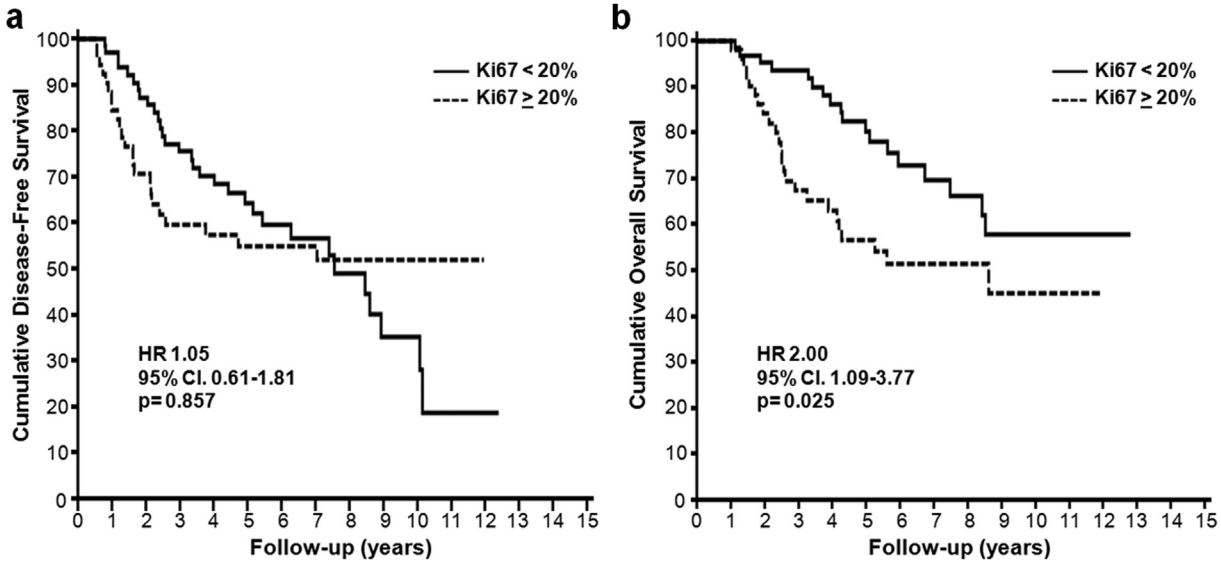

Fig. 5. Cumulative disease-free survival (a) and overall survival (b) stratified by Ki-67 index.

Table 4
Multivariate analysis.

Disease Free Survival	HR (95% CI)	*P value*
Neoadjuvant chemotherapy (Yes *vs* No)	1.92 (1.11–3.32)	***0.020***
PR (Negative *vs* Positive)	2.55 (1.48–4.38)	***<0.001***
Overall Survival		
Neoadjuvant chemotherapy (Yes *vs* No)	2.05 (1.08–3.92)	***0.029***
PR (Negative *vs* Positive)	2.23 (1.14–4.36)	***0.019***
Ki67 (≥20% *vs* < 20%)	2.03 (1.09–3.80)	***0.027***

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest statement

None.

Ethical approval

No ethical approval was required as this study is purely retrospective.

Acknowledgments

This work was supported by the Consorzio Interuniversitario Nazionale per la Bio-Oncologia (CINBO).

References

[1] Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol 2010;10(28):3271–7. http://dx.doi.org/10.1200/JCO.2009.25.9820.
[2] Cho SH, Jeon J, Kim SI. Personalized medicine in breast cancer: a systematic review. J Breast Cancer 2012;15:265–72. http://dx.doi.org/10.4048/jbc.2012.15.3.265.
[3] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC cancer staging manual. seventh ed. New York: Springer; 2010.
[4] Newman LA. Epidemiology of locally advanced breast cancer. Semin Radiat Oncol 2009;19:195–203. http://dx.doi.org/10.1016/j.semradonc.2009.05.003.
[5] Yu JI, Park W, Choi DH, Huh SJ, Nam SJ, Kim SW, et al. Clinical outcomes and prognostic factors of pathologic N3 breast cancer treated with modern standard treatments. Clin Breast Cancer 2015;15:512–8. http://dx.doi.org/10.1016/j.clbc.2015.06.004.
[6] Montero AJ, Rouzier R, Lluch A, Theriault RL, Buzdar AU, Delaloge S, et al. The natural history of breast carcinoma in patients with or >10 metastatic axillary lymph nodes before and after the advent of adjuvant therapy: a multi-institutional retrospective study. Cancer 2005;104:229–35. http://dx.doi.org/10.1002/cncr.21182.
[7] Duraker N, Caynak ZC, Bati B. Is there any prognostically different subgroup among patients with stage IIIC (any TN3M0) breast carcinoma? Ann Surg Oncol 2008;15:430–7. http://dx.doi.org/10.1245/s10434-007-9558-6.
[8] Lee JS, Kim SI, Choi SY, Park HS, Lee JS, Park S, et al. Factors influencing the outcome of breast cancer patients with 10 or more metastasized axillary lymph nodes. Int J Clin Oncol 2011;16:473–81. http://dx.doi.org/10.1007/s10147-011-0207-5.
[9] Basaran G, Devrim C, Caglar HB, Gulluoglu B, Kaya H, Seber S, et al. Clinical outcome of breast cancer patients with N3a (>10 positive lymph nodes) disease: has it changed over years? Med Oncol 2011;28:726–32. http://dx.doi.org/10.1007/s12032-010-9516-1.
[10] Koca E, Kuzan TY, Dizdar O, Babacan T, Sahin I, Ararat E, et al. Outcomes of locally advanced breast cancer patients with >10 positive axillary lymph nodes. Med Oncol 2013;30:615. http://dx.doi.org/10.1007/s12032-013-0615-7.
[11] von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012;30:1796–804. http://dx.doi.org/10.1200/JCO.2011.38.8595.
[12] Cameron DA, Anderson ED, Levack P, Hawkins RA, Anderson TJ, Leonard RC, et al. Primary systemic therapy for operable breast cancer–10-year survival data after chemotherapy and hormone therapy. Br J Cancer 1997;76:1099–105. http://dx.doi.org/10.1038/bjc.1997.514.
[13] Pierga JY, Mouret E, Laurence V, Diéras V, Savigioni A, Beuzeboc P, et al. Prognostic factors for survival after neoadjuvant chemotherapy in operable breast cancer. The role of clinical response. Eur J Cancer 2003;39:1089–96. http://dx.doi.org/10.1016/S0959-8049(03)00069-8.
[14] Angelucci D, Tinari N, Grassadonia A, Cianchetti E, Ausili-Cefaro G, Iezzi L, et al. Long-term outcome of neoadjuvant systemic therapy for locally advanced breast cancer in routine clinical practice. Cancer Res Clin Oncol 2013;139:269–80. http://dx.doi.org/10.1007/s00432-012-1325-9.
[15] Kuerer HM, Newman LA, Buzdar AU, Hunt KK, Dhingra K, Buchholz TA, et al. Residual metastatic axillary lymph nodes following neoadjuvant chemotherapy predict disease-free survival in patients with locally advanced breast cancer. Am J Surg 1998;176:502–9.
[16] Yildirim E, Semerci E, Berberoglu U. The analysis of prognostic factors in stage III-B non-inflammatory breast cancer. Eur J Surg Oncol 2000;26:34–8.
[17] Engstrom MJ, Opdahl S, Hagen AI, Romundstad PR, Akslen LA, Haugen OA, et al. Molecular subtypes, histopathological grade and survival in a historic cohort of breast cancer patients. Breast Cancer Res Treat 2013;140:463–73. http://dx.doi.org/10.1007/s10549-013-2647-2.
[18] Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000;406:747–52. http://dx.doi.org/10.1038/35021093.
[19] Normanno N, De Luca A, Carotenuto P, Lamura L, Oliva I, D'Alessio A. Prognostic applications of gene expression signatures in breast cancer. Oncology 2009;77(suppl 1):2–8. http://dx.doi.org/10.1159/000258489.

[20] Prat A, Ellis MJ, Perou CM. Practical implications of gene-expression-based assays for breast oncologists. Nat Rev Clin Oncol 2012;9:48–57. http://dx.doi.org/10.1038/nrclinonc.2011.178.
[21] Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160–7. http://dx.doi.org/10.1200/JCO.2008.18.1370.
[22] Nielsen TO, Parker JS, Leung S, Voduc D, Ebbert M, Vickery T, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. Clin Cancer Res 2010;16:5222–32. http://dx.doi.org/10.1158/1078-0432.CCR-10-1282.
[23] Prat A, Cheang MC, Martín M, Parker JS, Carrasco E, Caballero R, et al. Prognostic significance of progesterone receptor–positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol 2013;31:203–9. http://dx.doi.org/10.1200/JCO.2012.43.4134.
[24] Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer 2013. Ann Oncol 2013;24:2206–23. http://dx.doi.org/10.1093/annonc/mdt303.
[25] Maisonneuve P, Disalvatore D, Rotmensz N, Curigliano G, Colleoni M, Dellapasqua S, et al. Proposed new clinicopathological surrogate definitions of luminal A and luminal B (HER2-negative) intrinsic breast cancer subtypes. Breast Cancer Res 2014;16:R65. http://dx.doi.org/10.1186/bcr3679.
[26] Parker J, Prat A, Cheang M, Lenburg M, Paik S, Perou C. Breast cancer molecular subtypes predict response to anthracycline/taxane-based chemotherapy. Abstr 2019. In: San antonio breast cancer symposium; 2009 [San Antonio, TX].
[27] Albain KS, Barlow WE, Shak S, Hortobagyi GN, Livingston RB, Yeh IT, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. Lancet Oncol 2010;11:55–65. http://dx.doi.org/10.1016/S1470-2045(09)70314-6.
[28] Paik S, Tang G, Shak S, Kim C, Baker J, Kim W, et al. Gene expression and benefit of chemotherapy in women with node negative, estrogen receptor positive breast cancer. J Clin Oncol 2006;24:3726–34. http://dx.doi.org/10.1200/JCO.2005.04.7985.
[29] Viale G, Regan MM, Maiorano E, Mastropasqua MG, Golouh R, Perin T, et al. Chemoendocrine compared with endocrine adjuvant therapies for node-negative breast cancer: predictive value of centrally reviewed expression of estrogen and progesterone receptors-An International Breast Cancer Study Group. J Clin Oncol 2008;26:1404–10. http://dx.doi.org/10.1200/JCO.2007.10.6393.
[30] Early Breast Cancer Trialists' Collaborative Group (EGCTCG), Davies C, Godwin J, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 2011;378:771–84. http://dx.doi.org/10.1016/S0140-6736(11)60993-8.
[31] Dowsett M, Houghton J, Iden C, Salter J, Farndon J, A'Hern R, et al. Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. Ann Oncol 2006;17:818–26. http://dx.doi.org/10.1093/annonc/mdl016.
[32] Viale G, Regan MM, Maiorano E, Mastropasqua MG, Dell'Orto P, Rasmussen BB, et al. Prognostic and predictive value of centrally reviewed expression expression of estrogen and progesterone receptors in a randomized trial comparing letrozole and tamoxifen adjuvant therapy for postmenopausal early breast cancer: BIG 1-98. J Clin Oncol 2007;25:3846–52. http://dx.doi.org/10.1200/JCO.2007.11.9453.
[33] Dowsett M, Allred C, Knox J, Quinn E, Salter J, Wale C, et al. Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the Arimidex, Tamoxifen, Alone or in Combination Trial. J Clin Oncol 2008;26:1059–65. http://dx.doi.org/10.1200/JCO.2007.12.9437.
[34] Ellis MJ, Suman VJ, Hoog J, Lin L, Snider J, Prat A, et al. ACOSOG Z1031, a randomized phase 2 neoadjuvant comparison between letrozole, anastrozole and exemestane for postmenopausal women with ER rich stage 2/3 breast cancer: clinical and biomarker outcomes and the predictive value of the baseline PAM50-based intrinsic subtype-ACOSOG Z1031. J Clin Oncol 2011;29:2342–9. http://dx.doi.org/10.1200/JCO.2010.31.6950.
[35] Woodward WA, Vinh-Hung V, Ueno NT, Cheng YC, Royce M, Tai P, et al. Prognostic value of nodal ratios in node-positive breast cancer. J Clin Oncol 2006;24:2910–6. http://dx.doi.org/10.1200/JCO.2005.03.1526.
[36] Zhu C, Wu XZ. Proposal of new classification for stage III breast cancer on the number and ratio of metastatic lymph nodes. J Surg Oncol 2012;106:696–702. http://dx.doi.org/10.1002/jso.23118.
[37] Ito M, Moriya T, Ishida T, Usami S, Kasajima A, Sasano H, et al. Significance of pathological evaluation for lymphatic vessel invasion in invasive breast cancer. Breast Cancer 2007;14:381–7.
[38] Goldhirsch A, Glick JH, Gelber RD, Coates AS, Thürlimann B, Senn HJ, et al. Meeting highlights: international expert consensus on the primary therapy of early breast cancer 2005. Ann Oncol 2005;16:1569–83. http://dx.doi.org/10.1093/annonc/mdi326.
[39] Schoppmann SF, Bayer G, Aumayr K, Taucher S, Geleff S, Rudas M, et al. Prognostic value of lymphangiogenesis and lymphovascular invasion in invasive breast cancer. Ann Surg 2004;240:306–12.
[40] Harris LN, Ismaila N, McShane LM, Andre F, Collyar DE, Gonzalez-Angulo AM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American society of clinical oncology clinical practice Guideline. J Clin Oncol 2016;34:1134–50. http://dx.doi.org/10.1200/JCO.2015.65.2289.
[41] de Azambuja E, Cardoso F, de Castro G, Colozza M, Mano MS, Durbecq V, et al. Ki-67 as prognostic marker in early breast cancer: a meta-analysis of published studies involving 12,155 patients. Br J Cancer 2007;96:1504–13. http://dx.doi.org/10.1038/sj.bjc.6603756.
[42] Yerushalmi R, Woods R, Ravdin PM, Hayes MM, Gelmon KA. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol 2010;11:174–83. http://dx.doi.org/10.1016/S1470-2045(09)70262-1.
[43] Inwald EC, Klinkhammer-Schalke M, Hofstadter F, Zeman F, Koller M, Gerstenhauer M, et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry. Breast Cancer Res Treat 2013;139:539–52. http://dx.doi.org/10.1007/s10549-013-2560-8.
[44] Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009;101:736–50. http://dx.doi.org/10.1093/jnci/djp082.
[45] Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ, et al. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St. Gallen international expert consensus on the primary therapy of early breast cancer 2011. Ann Oncol 2011;22:1736–47. http://dx.doi.org/10.1093/annonc/mdr304.
[46] Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. PLoS Med 2010;7. http://dx.doi.org/10.1371/journal.pmed.1000279.
[47] Nordenskjöld A, Fohlin H, Fornander T, Löfdahl B, Skoog L, Stål O. Progesterone receptor positivity is a predictor of long-term benefit from adjuvant tamoxifen treatment of estrogen receptor positive breast cancer. Breast Cancer Res Treat 2016;160:313–22. http://dx.doi.org/10.1007/s10549-016-4007-5.
[48] Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, et al. Progesterone receptor modulates ERα action in breast cancer. Nature 2015;523:313–7. http://dx.doi.org/10.1038/nature14583.