Nodal Ratio as a Prognostic Factor in Patients with Four or More Positive Axillary Nodes Treated with Breast-conserving Therapy and Regional Nodal Irradiation

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Abstract. *Aim: This study aimed to investigate the prognostic value of the axillary lymph node ratio (i.e. positive axillary nodes to nodes removed) in patients with breast cancer treated with conservative surgery and regional radiotherapy. Patients and Methods: We retrieved the records of 195 patients with breast cancer with pathological stage pT1-2 pN2-3, treated from January 2005 to December 2013 at our Radiation Oncology Centers; their clinical data were retrospectively evaluated. All patients underwent lumpectomy or quadrantectomy with axillary lymph node dissection, adjuvant chemo-with/without hormonal therapy and irradiation to the whole breast and ipsilateral axillary apex, infraclavicular and supraclvicular nodes, excluding internal mammary nodes. The primary end-point was to evaluate the nodal ratio as a prognostic factor; moreover, the following prognostic factors were evaluated: age, biological status and molecular profile. Results: The median follow-up was 58 months (range=32-117.6 months). Two- and 5-year overall and recurrence-free survival rates were 96% and 88%, and 92% and 85%, respectively. On univariate analysis, factors influencing overall survival were nodal ratio >0.65 (p=0.033) and age (p=0.023); time to recurrence was detrimentally impacted only by Ki67 positivity ≥50% (p=0.049). At multivariate analysis, no significant associations were found. Conclusion: Adding irradiation to regional nodes after conservative surgery in patients with breast cancer with more than three positive axillary nodes does not alter the prognostic value of the nodal ratio, and we confirm this to be an important factor for predicting overall survival.*

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Breast cancer treatment necessitates a multidisciplinary approach, including surgery, radiotherapy and systemic treatment. The role of adjuvant radiotherapy has been well documented (1), and is today widely recognized. Adjuvant radiotherapy after breast-conserving surgery (BCS) has a positive impact on overall survival (2-4). Some studies have shown that women with poor locoregional disease control have a high risk of distant metastasis, resulting in a higher percentage of cancer-related deaths (5, 6). Therefore, it seems evident that a therapeutic approach that improves locoregional control might also increase the long-term survival; moreover, nodal recurrences confer a negative prognosis (7, 8).

It has been demonstrated that lymph-node status is the most important prognostic factor as a predictor of survival in breast cancer (9-11): patients with four or more positive lymph nodes have a worse prognosis compared to those having three or fewer histologically positive lymph nodes (12). Moreover, some authors support the use of the nodal ratio (NR), *i.e.* the number of positive nodes to the number of nodes excised, as a prognostic factor (13-15). Other prognostic factors include tumor volume, grading, receptor status, human epidermal growth factor receptor 2 (HER2)

expression and proliferative index (16): these factor allow patients with breast cancer to be classified according to risk classes (as luminal A, luminal B, HER2-overexpressing, and basal-like) (17).

Locoregional post-mastectomy irradiation is the standard treatment both for locally advanced breast cancer and in nodal-positive cases, leading to a 3- to 4-fold reduction in chest-wall recurrences and improving overall survival by 6% (18). However, some controversies exist regarding prophylactic irradiation of the axillary apex and supraclavicular nodes after BCS. Despite some authors having demonstrated no advantage of treating regional nodes in patients after BCS (19), some international guidelines recommend irradiation of these nodal areas when more than three positive nodes are detected (20).

The aim of this study was to investigate the impact of regional irradiation on the importance of NR as a prognostic factor after BCS in patients with more than three positive nodes.

Patients and Methods

Patients. We retrospectively evaluated the clinical records of patients with breast cancer who had undergone conservative surgery and axillary dissection with four or more positive nodes. From January 2005 to December 2013, 195 women with breast cancer were found to be eligible for inclusion in the present study. Patients were treated at three institutions (University Hospital of Messina, Taormina Hospital and Papardo Hospital). Only women with pT1-2 pN2-3 breast cancer treated by lumpectomy or quadrantectomy with axillary lymph node (levels I-II and/or III) dissection followed by whole breast and nodal irradiation were included in the cohort. All patients were staged according to the seventh edition of the American Joint Committee on Cancer TNM staging system for breast cancer (21).

Radiation treatment was performed with a mono-isocentric technique in all cases, with a delivered total dose of 50 Gy in 25 fractions, followed by an additional boost of 10-14Gy to the breast tumor bed, and 46-50 Gy in 23-25 fractions to ipsilateral axillary apex, infraclavicular and supraclavicular nodes. No radiation treatment was performed for internal mammary chain nodes.

Adjuvant systemic treatment was administered to all patients and consisted of chemotherapy with/without hormonal therapy and trastuzumab in HER2-positive cases, Data collected included NR, histological type and molecular features.

The positive rates for estrogen (ER) and progesterone receptor (PR) were determined by immunohistochemistry, and a value of ≥10% staining of cells was considered as positive. The proliferative activity was determined by immunostaining for the Ki67 antibody (Dako, Glostrup, Denmark). The fraction of proliferating cells was based on a count of at least 500 tumor cells. The Ki67 values were expressed as the percentage of positive cells in each case; on the basis of these values, the patients were divided into three groups, assuming cut-off values of <20% (low), ≥20% (medium) and ≥50% (high). The staining pattern of HER2 was classified into four groups: 3+: strong and diffuse staining; 2+: moderate and diffuse staining; 1+ focal staining >10% cancer cells; and negative. In our analysis, we only considered cases with 3+ immunostaining as HER2-positive. We then divided patients into molecular subtypes according to the 13th St Gallen International Breast Cancer Conference (2013) (17). Categorization of breast cancer subtype based on ER, PR, HER2 and Ki67 status was as follows: i) Luminal A (ER-positive or PR-positive, HER2-negative and Ki67-low); ii) luminal B (ER-positive or PR-negative, HER2-negative and Ki67-high); iii) HER2-positive or non-luminal (ER-negative, PR-negative, and HER2 overexpression or amplified); and basal-like or triple-negative (ER-negative, PR-negative, and HER2-negative). We set a cut-off value of NR at 0.65 for evaluating whether values higher or lower than this were associated with longer or shorter survival, respectively.

Table I. *Summary of demographic and clinical characteristics*.

Characteristic	Patients (n=195)
Mean± SD age (range), years	57.9±12.1 (29-83)
Histology, n (%)	
Ductal carcinoma	167 (85.6)
Lobular carcinoma	11 (5.6)
Other	17 (8.8)
Luminal, n (%)	
A	74 (37.9)
B	85 (43.6)
Basal-Iike	17 (8.7)
HER2 overexpression	4 (2.1)
Unknown	15 (9.7)
Chemotherapy, n (%)	
Anthracycline	112 (61.5)
Taxane	50 (27.5)
CMF	20 (11)
Endocrine therapy, n (%)	
Yes	188 (96.4)

The patients, tumors and treatment characteristics are summarized in Table I. Time to progression and overall survival were evaluated. This study received Institutional Review Board approval (approval number 38/2014).

Statistical analysis. All statistical analyses were carried out using the SPSS 21.0 software package (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Kolmogorov–Smirnov test. Univariate analysis was used to identify patient demographic and clinical characteristics that affected survival and recurrence. Survival time and time to recurrence were calculated from the date of starting radiation therapy and the Kaplan–Meier curves were generated to describe overall survival and time to recurrence. The Cox proportional hazards method was used to identify predictors for overall survival and recurrence. All statistical tests were two-tailed and a p-value of less than 0.05 was considered significant; p-values 0.05 or more but less than 0.10 were considered borderline significant.

Results

Patients and tumors characteristics. Table I summarizes patients and tumor characteristics. The average patient age was 57.9 (range=29-83) years. Most patients (85.6%) had

invasive ductal carcinoma. The average number of lymph nodes dissected was 18.5 (range=5-46), with a median of seven positive lymph nodes (range=4-35). A total of 137/195 patients (70.3%) had pN2 status, and 59/195 (30.3%) pN3. The median NR was 0.48 (range=0.14-1). ER and PR status was positive in 80.5% (157/195) and 73.3% of patients (143/195), respectively. Ki67 values were available in 72 cases (37% of all cases); HER2 (3+) was positive in 41 cases (21%). The molecular subtype was luminal A or B in the majority of cases (159/195, 81.5%); the molecular subtype was unknown in 15/195 (9.7%) of patients.

Overall, 182/195 patients (93.3%) received chemotherapy; 188/195 patients (96.4%) received hormonotherapy. The median follow-up time was 4.83 years (range=2.7-9.8 years).

General patient outcomes. Recurrences were identified in four patients (2%) and 13 patients (6%) died. There were two cases of supraclavicular recurrences (1%) and one breast ipsilateral recurrence (1%). Three patients (1.5%) had contralateral breast cancer, and three (1.5%) had other tumors (two colorectal, one uterine). Seventeen patients developed distant metastases (8.7%): 15 osseous, one hepatic and one pulmonary.

Two- and 5-year overall survival rates were 96% and 88%, respectively (Figure 1A). Two- and 5-year recurrence-free survival rates were 92% and 85%, respectively (Figure 1B).

Factors associated with patient outcome (overall survival and time to recurrence). At univariate analysis, higher age significantly reduced overall survival (p=0.027). NR>0.65 was associated with worse overall survival (p=0.033) (Figure 2). In addition, both HER2 (3+) overexpression and pN3 status were associated with reduced overall survival (p=0.062 and p=0.067, respectively), reaching borderline statistical significance. Conversely, in our cohort, time to recurrence was detrimentally affected only by a Ki67 value of 50% or more (p=0.049) (Figure 3). At multivariate analysis, no statistically significant associations were found for overall survival nor for recurrence.

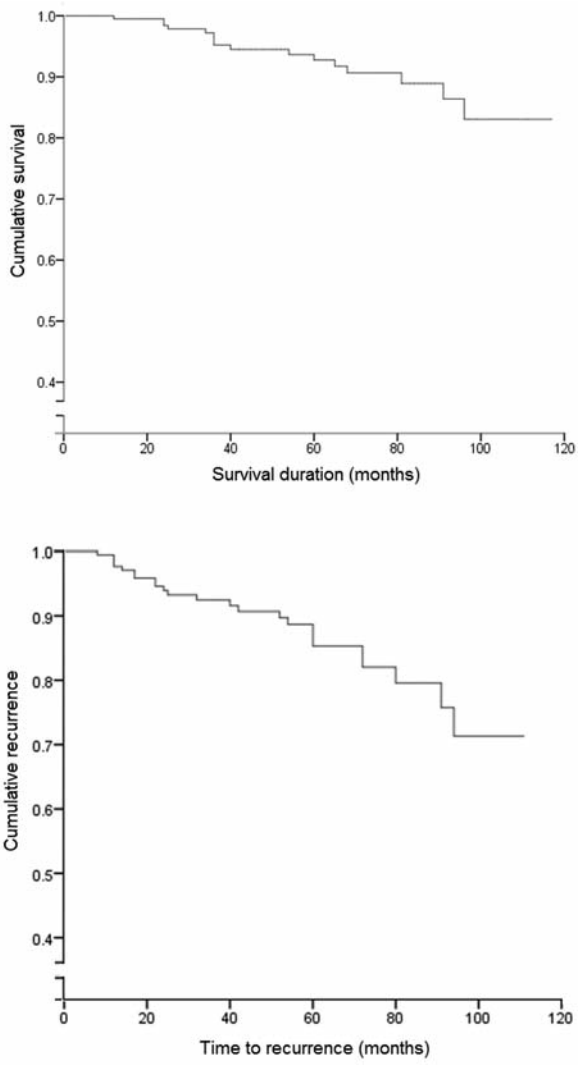

Figure 1. *Kaplan–Meier overall (upper panel) and recurrence-free (lower panel) survival curves of patients post radiotherapy after breast-conserving surgery for breast cancer.*

Discussion

Post-surgical radiation therapy including the chest wall and regional nodes was demonstrated to increase both recurrence-free and overall survival (1, 11). The presence of axillary lymph node metastases is the most important prognostic factor in patients with breast cancer (1, 9, 11) and the prognostic importance of the total number of excised nodes has also been recognized (22).

Some international guidelines recommend the use of supraclavicular irradiation when four or more positive axillary nodes are present (20). In contrast, some authors found no evidence of benefit associated with this treatment strategy, reporting supraclavicular nodal recurrences in patients with more than three positive axillary nodes in about 5.5% (19).

The absolute number of nodes involved is one of the three parameters evaluated in the Nottingham Prognostic Index, along with the tumor size and the histological grading (23). However, it has been shown by several authors that use of only the absolute number of involved lymph nodes is an inadequate prognostic indicator (24, 25). The NR proved to be an excellent predictor for survival, outcome or development of metastatic disease in node-positive patients with an adequate number of nodes removed (>13), that could be helpful in the management of patients (13-15). Our data confirm that the NR is indeed an important prognostic factor,

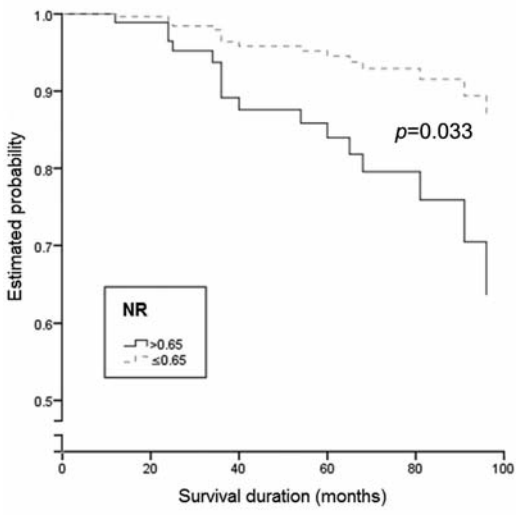

Figure 2. *Kaplan–Meier curve for overall survival according to the nodal ratio (NR) in months post-radiotherapy.*

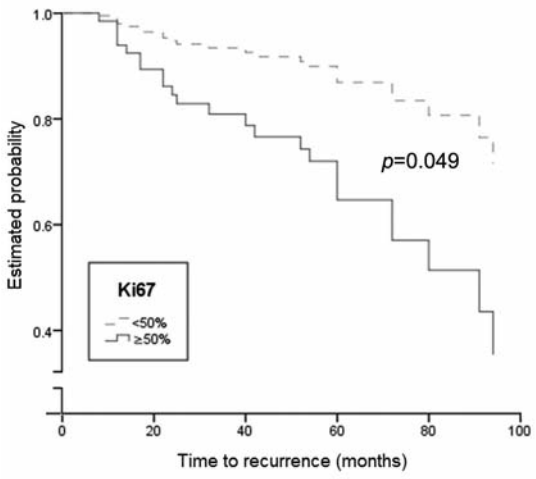

Figure 3. *Progression-free survival. Kaplan-Meier progression-free survival curve according to Ki67 positivity in patients after breast-conserving surgery for breast cancer in months post-radiotherapy.*

namely, that values greater than 0.65 are significantly associated with worse overall survival on univariate analysis.

A recent meta-analysis showed that the NR was a prognostic predictor for breast cancer, especially for clinically node-positive breast cancer, although the available evidence was unable to establish the most reliable cut-off point. Cut-off values of 0.2 and 0.65 have been considered suitable for predicting OS, DFS, BCCS and mortality in breast cancer (26).

Kim *et al*. studied 7,741 patients with N1 breast cancer and found that a NR greater than 0.18 predicted worse overall survival, and that adjuvant post-mastectomy radiotherapy should be recommended in this setting of patients (27). Tai *et al*. reviewed the data of 5,996 women from the Saskatchewan provincial registry and categorized the patients into three NR groups as: low (<25%), medium (>25% to <75%), and high (>75%), respectively. The authors demonstrated that for patients with more than 10 nodes examined, supraclavicular and axillary radiotherapy significantly improved the survival for the medium- and high-NR groups but not for the low-NR group (28). In our study, we found that a high NR is predictive of worse survival despite irradiation of the regional nodal areas. Our result is different from that of Tai *et al*., perhaps because they evaluated a heterogeneous series, including patients who had undergone mastectomy and lumpectomy. In our study, we noted that a high NR had no impact on the rate of locoregional recurrence. Conversely, Castrucci *et al*. found that in patients with four or more positive axillary nodes treated with traditional tangential/supraclavicular techniques, a high NR (>70%) predicted in-field regional recurrences (29). It should be noted that while all our patients underwent irradiation of the supraclavicular fossa, in the series of Castrucci *et al*., regional node radiation was delivered at discretion of the treating physician.

In our series, patients with pN3 status presented a poor overall survival with respect to those with pN2, reaching borderline statistical significance. This is in agreement with the study of Liao *et al*. (30), who found that NR and pN status are important prognostic factors with regard to overall survival for patients with node-positive breast cancer, but NR has demonstrated to possess an interesting prognostic value in the setting of patients who undergo BCS.

It is noteworthy that we recorded a low recurrence rate (1%) for the supraclavicular fossa, confirming that regional adjuvant irradiation seems to reduce the risk of recurrence in this anatomical region.

Age, T-status, ER/PR status, HER2 and Ki67 expression are other important prognostic factors that can predict the evolution of this disease (9, 16, 31, 32).

We found that increasing age resulted in lower overall survival (p=0.027). These data seem to confirm the results of the study by Dai Kubicky *et al*., who reported that older age (≥60 years) correlated with worse overall survival in patients with one to three positive lymph nodes (21).

In our cohort, the recurrence-free survival time was detrimentally impacted only by Ki67 ≥50%; although others also report this observation (33, 34), our series is too small to give this finding a clinical value.

An interesting aspect to consider from our analysis is that patients in the subset with HER2-positive phenotype seem to have a poor prognosis with respect to the other categories. Moreover, the patients with basal-like disease seem to have

an overall survival similar to those with luminal A or B breast cancer. This is in contrast with the results of the St. Gallen conference, which underlined that the category of patients with the best prognosis has a luminal A molecular phenotype; conversely, the basal-like group had poorer prognosis, although the effect weakened at longer follow-up (35). This result is probably a result of the small number of patients of the basal-like and, particularly, HER2-positive subsets.

In conclusion, we note that the NR value, in patients submitted to BCS with more than three positive axillary nodes and regional irradiation, may have an important role as a predictive prognostic factor. Further studies are required to investigate if a more aggressive axillary approach (surgery and irradiation) could modify the observations made on this issue.

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The Authors have no disclosures and conflict of interest

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