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Original article

Predictive factors of isolated tumor cells and micrometastases in axillary lymph nodes in breast cancer

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ABSTRACT

Introduction: Since the introduction of the sentinel lymph node biopsy (SLNB) in patients with breast cancer, micrometastases and isolated tumor cells are detected frequently in the SLN. As such, they offer an opportunity to study the development of regional metastases in breast cancer.
Patients and methods: Between June 1999 and November 2010 1418 patients with cT1-2N0 breast cancer underwent SLNB. Primary tumor characteristics and information regarding regional lymph node involvement were collected prospectively. Patients were categorized into four levels of lymph node involvement: pN0, pN0(i+), pN1mi and pN $\geq$ 1a. An univariate analysis and a binary logistic regression analysis were performed to assess the relation between patient- and tumor characteristics and lymph node involvement.
Results: Increasing tumor size and younger age were associated with a higher risk of pN1mi and pN $\geq$ 1a and a lower chance of pN0 and pN0(i+). Triple negative molecular subtype was associated with a decreased risk of pN1mi and pN $\geq$ 1a. Tumor size was positively related to overall occurrence of regional lymph node metastases in a linear manner.
Conclusion: Patients with larger tumors, no triple negative disease, and younger age showed a decreased chance of both pN0 and pN0(i+) and an increased risk of both pN1mi and pN $\geq$ 1a. There seems to be a gradual shift in risk pattern from pN0 to pN0(i+) to pN1mi and to pN $\geq$ 1a-disease. The presence of the smallest metastases remained fairly constant over time when compared to macrometastases. This constant presence suggests that the risk of seeding and outgrowth of metastases remains constant over time.

Introduction

Since the introduction of the sentinel lymph node biopsy (SLNB) micrometastases and isolated tumor cells are detected more often in patients with breast cancer. This limited metastatic lymph node involvement is observed in more than ten per cent of all patients, and affects approximately 40 per cent of patients with lymph node metastases.[1] Controversy remains about the clinical impact of these small metastases. Some[2] suggest that patients with isolated tumor cells and micrometastases have a significantly worse prognosis than patients with pN0-disease, while others did not observe a significant prognostic effect.[3–5] The question remains whether lymph node micrometastases should be considered as pN0-disease or as pN1-disease.

As the above studies are not easy to interpret due to almost exclusively non-randomized, i.e. observational, designs in which the confounding effects of adjuvant treatment cannot be excluded, other investigations into the biology of breast cancer with limited regional lymph node involvement are needed. With respect to the presence of macrometastatic lymph node involvement there is a well established proportional increased risk of regional lymph node metastases with increasing primary tumor size.[6] Other known predictors of axillary lymph node macrometastases are age, lymphovascular invasion (LVI) and histological subtype.[7]

To our knowledge, risk factors for the occurrence of pN0(i+) and pN1mi have not been studied before. Here we report how established risk factors for macrometastatic regional lymph node

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involvement relate to the risk of such limited regional lymph node involvement in a large cohort of Dutch breast cancer patients, and whether these relations are more in agreement with associations for pN0-disease or for macrometastatic disease.

Patients and methods

Patients and clinical procedure

After approval of the institutional ethical committee, data were collected regarding all patients who were operated for cT1-2N0 breast cancer and in whom SLNB was used as a staging procedure between June 1999 and November 2010 in the Diakonessenhuis Utrecht hospital. During the study period 1418 patients underwent surgical treatment. A diagnosis of invasive breast cancer was established preoperatively. Patients who had a history of ipsilateral breast cancer were excluded since lymph node metastases might originate from the previous tumor. Also, patients with a multifocal or a multicentric tumor were excluded since the origin of eventual lymph node tumor involvement cannot be ascribed to one specific lesion in these patients. Therefore, it is impossible to establish a relation between lymph node involvement and the primary tumor characteristics in the latter patients.

Lymphoscintigraphy was performed on the day of surgery. Visualized axillary and internal mammary SLNs were excised.[8] If no axillary SLN was visualized, the axilla was explored for patent blue containing SLNs. If the axillary SLN contained metastases an axillary lymph node dissection (ALND) was performed either during the same operation following frozen section analysis or as a second procedure after definitive pathology. Patients with isolated tumor cells (pN0(i+)) in the SLN did not receive a routine ALND. In addition, a number of patients participated in the AMAROS trial[9] (ALND versus radiotherapy on the axilla in patients with metastases in the axillary SLN). Patients allocated to the radiotherapy-arm of the latter study did not undergo a complementary ALND if the SLN contained metastases.

At the pathology department, axillary SLNs were bisected, formalin fixated, paraffin embedded and both halves were cut at 5 levels 250 μm apart. All cuts were evaluated with H&E and immunohistochemically with antibodies to cytokeratin-8 in line with the national pathology guideline.[10] Lymph nodes from the complementary ALND specimens were halved and one slice from both halves was examined with H&E.

Pathological diameter, malignancy grade based on the grading system by Elston and Ellis[11] and estrogen (ER) and progesterone (PR) receptor status of the primary tumor were collected prospectively. ER and PR status were tested by immunohistochemistry on formalin-fixated and paraffin embedded tumor tissue in concordance with the Dutch guideline[10] and were considered positive in case of >10% positive tumor cells.[12] Her2 receptor status was routinely examined since 2004 and analysis by CISH (chromogenic in situ hybridization) was used since 2007. Metastatic lymph node involvement was classified according to the 6th edition of the UICC TNM classification.[13]

Analysis

We studied the relation between age, primary tumor size, modified Bloom and Richardson grade, and ER, PR and Her2 receptor status with the extent of regional lymph node involvement, specifically focusing on pN0(i+) and pN1mi. For this purpose, we categorized patients into four levels of regional lymph node metastasis: pN0, pN0(i+), pN1mi and pN ≥ 1a.

First, we calculated the absolute risk of each level of lymph node involvement according to patient and tumor characteristics univariably. Continuous variables were categorized into four subgroups for this analysis (age into approximate quartiles and tumor size in 4 cm increments up to ≥3 cm). Furthermore, besides evaluating ER, PR and Her2 receptor status separately, we combined the receptor expressio! data into three distinct molecular subtypes resembling Perou's breast cancer subtypes: 1) ER or PR positive and Her2 negative, 2) Her2 positive, and 3) ER, PR and Her2 negative (triple negative). We report risk estimates with corresponding 95% confidence intervals (95%CI) based on the Wilson Score method. Differences between patient and tumor characteristics and level of lymph node involvement were tested for statistical significance by Chi-squared tests.

To investigate the independent relation of patient and tumor characteristics with level of lymph node involvement we used binary logistic regression analysis. Four separate analyses were done, one for each level of lymph node involvement and each time comparing the level of interest with all other levels (e.g. pN0 versus pN0(i+), pN1mi and pN ≥ 1a combined). Each logistic regression analysis first related all risk factors with the outcome univariably and then multivariably using all variables in one model. Risk factors included in these models were: tumor size (above categories), age (continuous), modified Bloom and Richardson grade (good, intermediate, poor) and molecular subtypes (above categories), using the first level of each categorical variable as the reference. We report odds ratios (OR) and corresponding 95%CI and tested model fit by the Hosmer–Lemeshow test and found no violation for any of the models.

As not all patients had complete data for all variables, we used multiple imputation (5 datasets) prior to the logistic regression analyses. Data were missing for the following variables: modified Bloom Richardson grade ($n = 15$ of the analysis dataset), ER status ($n = 3$), PR status ($n = 4$), Her2 receptor status ($n = 144$), and consequently molecular subtype ($n = 145$). Multiple imputation was based on logistic regression models using all variables described above, including the level of lymph node involvement as well as information on palpability and histological subtype as predictors. This way, one or more variables were imputed for 156 patients (12% of analysis dataset). However, we also analyzed the data using a complete-case approach to evaluate the influence of the multiple imputation. Proportional degrees of metastatic lymph node involvement were plotted against tumor size, assuming primary tumor size to be an adequate surrogate for time. All analyses were performed using SPSS for Windows (PASW Statistics 18.0; SPSS, Chicago, Illinois).

Results

After exclusion of 65 patients with multifocal or multicentric disease and 20 patients with a history of ipsilateral breast cancer the study group consisted of 1333 patients. Median age of the 1333 patients was 59.9 years (range 24.2–92.0). Baseline characteristics are shown in Table 1. One or more SLNs were visualized on lymphoscintigraphy in 1321 patients (99.1%): 1043 patients had axillary SLNs, 8 patients had internal mammary (IM) SLNs and 270 had IM and axillary SLNs. SLNs were retrieved in 1325 (99.4%) patients; axillary SLNs were retrieved in 1099 patients, IM SLNs in 7 patients and in 219 patients axillary and IM SLNs were removed.

Lymph node metastases were detected in 517 patients: axillary metastases in 499 patients of whom 55 patients also had IM metastases; 18 patients had IM metastases only. Complementary ALND was omitted in 102 patients with involved axillary SLNs; based on the examination of the SLN these patients were classified as pN0(i+) $n = 43$; pN1mi $n = 35$ and pN1 $n = 24$. Reasons for omitting ALND in the 24 patients with macrometastatic disease were: metastatic lymph node in breast tissue or Rotters fat ($n = 2$),