Influence of receptor status and proliferation index in skeletal metastases of breast carcinoma on pathological fracture occurrence

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Abstract

Purpose. Bones affected with metastatic breast carcinoma are prone to pathological fracture, impairing quality of life and survival rate. This study examined estrogen, progesterone, and HER2 receptors status, and Ki-67 index in skeletal metastases of breast carcinoma and their potential influence on pathological fracture occurrence.

Methods. The study included 152 samples of skeletal metastasis of breast carcinoma, each obtained from an individual patient. Allred's score was used for immunohistochemical interpretation. Clinical and radiological data were gathered from each patient's history of the disease.

Results. Femur stood out as an independent risk factor – it had a nine times higher chance for pathological fracture than other bones. Estrogen receptors, HER2, and Ki-67 status had no statistical significance for pathological fracture occurrence. Progesterone receptors showed significance for fracture development. The probability of fracture in progesterone receptor positive metastases was 68.1%, risk increasing with the rise of positive cells percentage, regardless of the expression intensity.

Conclusion. Receptor status is important for skeletal metastases of breast carcinoma and can be used in clinical decision-making for systemic therapy and surgical treatment in fracture prevention.

1. Introduction

The most frequently diagnosed cancer in the female population is breast carcinoma (BC). Dissemination in bones represents a natural course of the disease, and in 65-75% of patients, skeletal metastases (SM) will occur. In 5-6% of patients, SM are confirmed at the time of the first diagnosis of BC. [1-4] The most important complication of SM is a pathological fracture which impairs the quality of life and affects the survival rate. [5, 6] It is well known that the status of estrogen receptor (ER) and progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki67 proliferative index are crucial for the effectiveness of breast carcinoma therapy. [7-10] Determination of these factors is mandatory in primary BC biopsy analysis, while it was rarely used for metastatic lesions. [11-15] Recent studies investigated receptor status in SM, reporting possible differences between them and primary tumors. [16-19] Data on the influence of receptor status in metastases and their biological behavior and consequent clinical appearance is scarce. The aim of this study is to determine ER, PR, HER2 status, and Ki67 index in SM of BC and their possible impact on pathological fracture occurrence.

2. Material And Methods

Medical records at the Referent Center for Bone and Soft Tissue Oncology of the Institute for Pathology, Medical Faculty, University of Belgrade were retrospectively reviewed in the ten years period (June 2011.-June 2021.), and samples with SM of BC were identified. The study included 152 samples, each obtained from an individual patient. Only samples with completely determined ER, PR, HER2, and Ki67 status were included. Indeterminate result samples were excluded. Only female patients surgically treated at Institute
for Orthopaedics “Banjica” in Belgrade were included in the study. Patients treated elsewhere, and male patients were excluded. Clinical and radiological data were obtained from each patient’s history of the disease at Institute “Banjica.”

2.1 Immunohistochemistry

Serial sections, 5 µm thick, were cut for immunohistochemical (IHC) analysis of ER, PR, HER2, and Ki67 using US Food and Drug Administration approved primary rabbit monoclonal antibodies (Ventana Medical Systems, Oro Valley, Arizona, USA; ER [6F11 clone], PR [1E2 clone], HER2 [4B5 clone], Ki-67 [M7240 Clone MIB-1, dilution 1 : 100; Dako]). Positive and negative controls were included for each case. The slides were evaluated according to the ASCO/CAP guidelines. [20, 21] The Allred score was calculated for ER and PR by adding the percentage and intensity of positively stained tumor cells for a total score ranging from 0 to 8. The percentage of positive tumor cells was divided into 6 categories, 0 - negative, 1 indicating <1%, 2 - 1%-10%, 3 - 11%-33%, 4 - 34%-66%, and 5 - 67%-100% (Fig. 1). The intensity of positive tumor cells was averaged across the predominant area and scored 0 indicating no staining, 1 indicating weak staining, 2 indicating moderate staining, and 3 indicating strong staining. HER2 slides were analyzed for the intensity of staining and percentage of stained cells and classified as negative (score 0 or 1+), equivocal (score 2+), or positive (score 3+). For equivocal cases, IHC was repeated, and if still unchanged, FISH was performed. Tumor cells with nuclear staining were considered positive for Ki-67, and we reported as a number of positive in 100 counted cells.

2.2 Statistical analysis

Statistical analysis was performed using SPSS v.28.0 software (SPSS Inc., Chicago, IL, USA). Descriptive data are expressed as a percentage of a group for discrete measures. The Pearson's chi-squared test was used to analyze all categorical data. A normal distribution of all numeric data was tested using the Kolmogorov-Smirnov test. If the data was non-parametric, it was analyzed using the Mann-Whitney test. The association between observed parameters and the pathological fracture was analyzed by univariate and multivariate binary logistic regression. The probability of positive and negative PR patients in the examined groups (with and without pathological fracture) is expressed as a negative predictive value (NPV) and positive predictive value (PPV) with 95% confidence intervals (CIs). For all statistical analyses, p<0.05 was considered significant.

2.3 Ethics

The study was approved by the Ethical Committee of the Medical Faculty, University of Belgrade (1322V-3), and the research was carried out in compliance with the Declaration of Helsinki. It was conducted retrospectively as data analysis of an existing data bank without any additional experiment on human tissue.

3. Results
3.1 Demographic characteristics

The mean age of the patients was 61.4 years, ranging from 33 to 83 years. Distribution was: <40 y. 5 pts, 41-50 y. 19 pts, 51-60 y. 42 pts, 61-70 y. 51 pts, 71-80 y. 32 pts and >81 y. 3 pts. Duration of symptoms related to SM varied from 1 day (in patients with sudden pathological fractures and no previous knowledge of breast cancer) up to 60 months, mean 4.4 months, SD=8.2. The mean period from diagnosis of the primary tumor to the diagnosis of SM was 49.5 months, SD=61.5.

3.2 Skeletal localization and pathological fracture

Bone the most frequently affected was femur in 71 patients (46.7%). Twenty-four patients (33.8%) had metastasis in the femoral neck, 27 (38%) in trochanter, 19 (26.8%) in the diaphysis, and one (1.4%) in condyles.

A total of 91 (59.9%) pathological fractures were identified. Fifty-five (60.4%) of all fractures were in the femur. This number represents 77.5% of all femurs with metastases in contrast to 16 femurs (22.5%), in which metastases were not associated with fracture (p<0.01). All the patients with metastases in the femoral neck had a pathological fracture at this site; 20 (74.1%) of 27 metastases in the trochanteric region were fractured, and 11 (57.9%) of 19 in the diaphysis (Fig.2).

3.3 Immunohistochemistry

One hundred and seventeen (76.9%) biopsy samples were positive to ER (ER+), 72 (47.3%) to PR (PR+), and 57 (37.5%) to HER2 (HER2+). Seventy-one (60.7%) of ER+ metastases and 32 (56.1%) of HER2+ metastases were associated with pathological fracture. The incidence of pathological fracture was not statistically significant between ER+ and ER- metastases (p=0.708), also as between HER2+ and HER2- (p=0.468). The median Ki67 value was 20, ranging from 1 to 90, identical in metastases with and without fracture. Ki67 proliferative index showed no statistical significance for the fracture risk (p=0.542).

Opposite to these markers, PR expression in tumor cells was significantly associated with pathological fractures. Forty-nine (68.1%) of PR+ metastases were in bones with pathological fracture showing marginal statistical significance (p=0.051). We analyzed the significance of each PR score along with its parameters - intensity of expression (0-3) and percentage of positive cells (0-5) (Table 1). The mean value of PR score in metastases without fracture was 2.02, SD=2.81, and in metastases with fracture 3.02, SD=3.14 (p<0.05). The staining intensity did not show the significance for fracture risk (p=0.90), but a percentage of positive cells did (p<0.05).
Table 1. – PR scores in bones with and without pathological fracture

<table>
<thead>
<tr>
<th>PR score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (% of total)</td>
<td>78</td>
<td>2</td>
<td>2</td>
<td>13</td>
<td>9</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>15</td>
<td>152</td>
</tr>
<tr>
<td>Number of patients with fractures (%)</td>
<td>(51,3)</td>
<td>(1,3)</td>
<td>(1,3)</td>
<td>(8,6)</td>
<td>(5,9)</td>
<td>(7,9)</td>
<td>(6,6)</td>
<td>(7,2)</td>
<td>(9,8)</td>
<td></td>
</tr>
<tr>
<td>Number of patients without fractures (%)</td>
<td>40</td>
<td>2</td>
<td>1</td>
<td>10</td>
<td>6</td>
<td>7</td>
<td>6</td>
<td>6</td>
<td>13</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>(51,3)</td>
<td>(100)</td>
<td>(50)</td>
<td>(76,9)</td>
<td>(66,6)</td>
<td>(58,3)</td>
<td>(60,0)</td>
<td>(54,5)</td>
<td>(86,7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>61</td>
</tr>
<tr>
<td>Number of patients without fractures (%)</td>
<td>(48,7)</td>
<td>(0)</td>
<td>(50)</td>
<td>(23,1)</td>
<td>(33,4)</td>
<td>(41,7)</td>
<td>(40,0)</td>
<td>(45,5)</td>
<td>(13,3)</td>
<td></td>
</tr>
</tbody>
</table>

We determined positive (PPV) and negative predictive values (NPV) for each of the receptors (Table 2). The PPV for fracture in PR+ tumors is 68.1%.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>NPV</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>0.429 (0.263-0.606)</td>
<td>0.607 (0.512-0.696)</td>
</tr>
<tr>
<td>PR</td>
<td>0.475 (0.362-0.590)</td>
<td>0.681 (0.560-0.786)</td>
</tr>
<tr>
<td>HER2</td>
<td>0.379 (0.281-0.484)</td>
<td>0.561 (0.424-0.693)</td>
</tr>
</tbody>
</table>

Table 2. – PPV and NPV for ER, PR and HER2 receptors

Finally, fracture incidence in femurs with PR+ SM (86.8%) was higher compared to the incidence of fractures in other bones with PR- SM (31.9%) (p <0.01).

4. Discussion

The natural course of BC leads to its spreading and SM development. [1, 3, 10, 22] Pathological fractures are a common complication in patients with these metastases. [1, 18, 19] Previous studies described various risk factors for pathological fracture occurrence. [5, 18, 23] In our study, fractures were verified in
59.9% of patients. Such a high fracture rate could be due to a large number of postmenopausal women, especially in the late postmenopausal period older than 70 years. It is well known that such age distribution implies a high risk for fractures, particularly at the level of the femoral neck. [24, 25] The association of these factors (age and metastatic disease) is expected to increase the likelihood of fractures.

The femur was affected in 46.7% of patients, and 60.4% of all fractures were in the femur. Furthermore, 77.5% of SM in the femur were associated with pathological fractures. [26] An important finding in the study was that every patient with femoral neck metastasis had a pathological fracture at this site, also as 74.1% of patients with trochanteric metastases. All this indicates the necessity of an early and continuous radiological observation of the skeletal system, especially the hip joint.

Studies showed that ER and PR status in BC tissue influence the SM development (localization in axial or appendicular skeleton, radiographic appearance as sclerotic or lytic). [15, 17–19, 27–29] Papers described an influence of receptor status on patient survival after pathological fracture and outcome of surgical treatment [26, 30] but, to our knowledge, no study investigated the influence of receptor status on pathological fracture occurrence.

In this study, statistical significance for pathological fracture was found in the expression of the PR. Among the analyzed samples, 47.3% were PR positive, and 68.1% had a pathological fracture. PR positive patients had a PPV for developing a pathological fracture of 68.1%. Moreover, we found that the association between PR and fracture occurrence depends on the percentage of the stained cells and not on the staining intensity, the risk for fracture increasing with the rise of positive cells percentage, regardless of the expression intensity. Beside triple-negative primary tumor, which is known to be the most aggressive, PR-positive primary breast carcinoma, shows more aggressive features in comparison to ER-positive carcinoma. [31–34] Research shows that this, as well, is the case with SM, and the significance of this study is that it provides prognostic data useful for possible early surgical treatment and fracture prevention.

5. Conclusion

Presented data emphasize the importance of receptor status determination in SM of BC. Particular attention should be directed towards PR receptors. Regardless of their intensity of expression, any PR receptor positivity should be considered as an increased risk for fracture, especially in the femur. The findings should be regarded with caution because this is a retrospective study with confounding factors such as prior chemo and radiotherapy.

Declarations

FUNDING

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COMPETING INTERESTS

The authors declare that they have no conflict and competing interests. All the authors of this paper are in accordance with the principles of publication, and they have no benefits in any form, directly or indirectly, to the subject of this article.

DATA AVAILABILITY

The datasets generated during and analyzed during the current study are not publicly available due to patients’ information confidentiality and policies of our Institutions but are available from the corresponding author on reasonable request.

CODE AVAILABILITY

No code was used in this study.

AUTHOR CONTRIBUTIONS

SR was responsible for designing and writing the protocol, conducting the search, collecting surgical data, reviewing other studies in this field, extracting and analyzing data, interpreting results, and writing the report. LS was responsible for pathologic diagnostics, collecting pathological data, analyzing and interpreting results, and writing the report. GD took part in collecting and analyzing radiological data, analyzing and interpreting results. DD contributed to collecting pathological data and took part in analyzing and interpreting results. LM helped collecting surgical data, reviewing studies at writing the report. BM conducted the statistical analysis and helped analyze and interpret the results. JS took part in designing and writing the protocol, supervising pathological data, reviewing previous studies, analyzing and interpreting results.

Reference


Figures
Figure 1

a. Typical morphology of skeletal metastasis of breast carcinoma (H&E, 4x), b. Bone trabeculae surrounded by tumor tissue (H&E, 20x) c-h: Percentage of PR positive tumor cells was divided into 6 categories (c: 5/ indicating 67%-100%; d: 4/ 34%-66%; e: 3/ 11%-33%; f: 2/ 1%-10%, g: 1/ <1%, h: 0/ negative)(PR,20x)
Figure 2