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EVALUATION OF BONE METASTASIS OF BREAST CANCER TO LONG OR SHORT BONES, ACCORDING TO MOLECULAR SUBTYPES: RETROSPECTIVE STUDY

Avaliação da metástase óssea do câncer de mama em ossos longos ou curtos, segundo os subtipos moleculares: estudo retrospectivo

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ABSTRACT

Bone is the most frequent site for breast cancer metastasis. Identifying the possible preference of bone metastasis, such as long or short bones, according to molecular subtypes, could alter oncologists approach, paying special attention to these particular group of patients reducing the side effects of the bone metastatic process, involving multidisciplinary team with orthopedists, minimizing possible sequelae of this metastatic process. Detecting different metastatic sites to long or short bones, according to the molecular subtypes and their possible correlation. Fifty-eight patients with only bone metastasis were chosen. The study material was obtained from paraffin embedded primary tumors. Statistical analysis of the data was carried out. The luminal A, luminal B, hybrid luminal, HER2 + and triple-negative / basal-like molecular subtypes were identified. The molecular subtypes compared to the age of bone implants, the distribution of bone implants, and the disease free interval were not statistically significant.

KEYWORDS: Molecular biology; breast cancer; neoplasm metastasis.

RESUMO

Acometimento ósseo é o sítio mais comum de metástase do carcinoma de mama. A identificação de possível preferência conforme os subtipos moleculares, na precocidade ou no acometimento de ossos longos ou chatos, poderia alterar a prática médica de oncologistas, dirigindo especial atenção a esses grupos de pacientes e suas possíveis complicações, em atendimento multidisciplinar com ortopedistas, minimizando possíveis sequelas desse processo metastático. Detectar a instalação dos diferentes sítios metastáticos para ossos longos ou chatos (curtos), conforme os subtipos moleculares e sua possível correlação. Foram selecionados 58 casos de pacientes com câncer de mama que apresentaram exclusivamente metástases ósseas. O material de estudo foi obtido dos tumores primários emblocados em parafina. Realizaram-se análises estatísticas dos dados. Foram identificados os subtipos moleculares luminal A, luminal B, luminal híbrido, HER2+ e triplo-negativo/basal like. Os subtipos moleculares comparados com a idade de implantes ósseos, a distribuição de implantes ósseos e o intervalo livre de doença não mostraram significância estatística.

PALAVRAS-CHAVE: Biologia molecular; neoplasias da mama; metástase neoplásica.

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INTRODUCTION

Bones represent the most common site of distant metastasis of breast carcinoma. Bones from different parts of the skeleton, especially short (flat) bones, are often compromised by metastatic dissemination in women with breast cancer. It is not well understood why the initial mechanism of metastatic implants has a greater preference for bones. Among the short (flat) bones, the sequence of impairment for sternum, ribs, vertebrae and pelvis is observed. Short (flat) bones are affected before long ones[1].

Bone metastasis is strongly associated with positive estrogen receptor/negative progesterone receptor in tumors. Significant difference in tumors with estrogen receptor expression, between high and low grade with bone metastasis, suggests that different panels of molecular markers could be used to predict bone metastasis in these two groups of tumors[2].

The average time to diagnosis of only breast cancer metastasis from the last follow-up or death was 55.2 months. Only bone metastasis have been reported to occur in 17-37% of patients with distant metastasis. Metastasis to the confined distance to the skeleton presents a more favorable prognosis than other types of distant metastasis or multiple metastasis to bones and viscera. Other investigators reported that the median survival of patients with bone metastasis alone was 24-54 months. The favorable feature of the primary tumor accounts for the modest prognosis of women with only bone metastasis[3].

There is great evidence on the differences in dissemination among the biological subtypes of breast cancer. A study performed to analyze the metastatic pattern according to the biological subtype explores the corresponding prognosis. Biological subtype was defined by immunohistochemistry according to the criterion of St. Gallen, 2013, Swiss city where annual meetings of oncologists occur, in which consensus of prognoses and treatments are constructed, as adapted in Table 1. Association between biological subtypes and the distant and different locations were analyzed. Result was reported by taking luminal A from breast carcinoma as a reference. Triple-negative breast cancer demonstrated large tropism for lung, while the non-luminal subtype human epidermal growth factor type 2 (HER2) was associated with high rate of liver metastasis. All subtypes were associated with low risk of bone only location. Briefly, this study added information to understand the complexity of breast cancer and its clinical manifestations. It also proposes categorization between different subgroups based on the immunohistochemical resources, as it could predict the preferential anatomical site of the first distant metastasis, as well as specific prognosis. It is therefore tempting to hypothesize some practical implication in terms of "adapted" management, i.e., surveillance protocols and/or therapeutic strategies that need to be verified by clinical trials[4].

Table 1. Immunophenotypic profile to approximate molecular classification in breast carcinoma.

Molecular subtype	Profile of biomarkers
Luminal A	RE+ and/or RP+; HER2-; Ki-67<14%
Luminal B	RE+ and/or RP+; HER2-; Ki-67≥14%
Luminal hybrid	RE+ and/or RP+; HER2+
HER2+	RE-; RP-; HER2+
Triple negative	RE-; RP-; HER2-
Basal like	RE-; RP-; HER2-; CK 5/6+ and/or EGFG+

Source: Hammond et al.[10]; Cheang et al.[11]; Wolff et al.[12]; Wludarski and Bacchi[13]; Cheang et al.[14]; Bhargava et al.[15].
ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor type 2; Ki-67: protein encoded by the MKI67 gene; EGFG: epidermal growth factor gene.

Differences in the biological characteristics of breast cancer can be explained by differences in the pattern of changes between genes that act on carcinogenesis. Several studies have been conducted to determine the value of genetic changes as prognostic markers for these patients. The molecular prognostic markers used in clinical practice are: estrogen receptor (ER), progesterone receptor (PR) and tyrosine kinase receptor (ERBB2 / HER2). The presence or absence of these proteins is commonly detected using immunohistochemistry analysis. Thus, three main molecular classes were established: positive hormone receptor tumors, HER2 positive tumors and negative tumors for all the markers used. These classes have been integrated into diagnosis and treatment and help to stratify the risk of recurrence, especially in lymph node negative patients[5].

Involvement of axillary lymph nodes is considered the most informative prognostic factor. In practice, patients with four or more positive lymph nodes are considered a subgroup of unfavorable prognosis[6]. In the year 2000, Perou et al. published a work that became a reference to classify breast cancers in molecular subtypes, according to the gene expression pattern: luminal A, luminal B, superexpressor HER2, basaloid and normal-like[7].

According to Barros and Leite, these tumor subgroups present varying patterns of behavior regarding the expression of genes, the rate of tumor growth, as well as prognosis and sensitivity to treatment. According to these authors, the luminal subtype A corresponds to 30-40% of the cases; luminal B, 20 to 30%; and HER2 and basaloid, from 15 to 20% of the sample[8].

OBJECTIVE

To detect the installation of different metastatic sites for long or flat (short) bones in breast cancer, according to the molecular subtypes and their possible correlation.

METHOD

This study is a historical cohort, in which 58 cases of invasive breast carcinoma, exclusively affected by bone metastasis, attended by the Department of Mastology of *Hospital Amaral Carvalho*, Jaú, São Paulo, were retrospectively selected between January 2000 and January 2012. The present study was approved by the Research Ethics Committee of the *Hospital Amaral Carvalho* and the *Plataforma Brasil*, under No. 1.546.684, dated May 16th, 2016.

Patients with breast cancer exclusively presenting bone metastasis from breast carcinoma; who underwent immunohistochemistry and adjuvant chemotherapy, according to the protocol of the Clinical Oncology Department of *Hospital Amaral Carvalho*; with adjuvant radiotherapy treatment, if indicated; with hormone therapy with tamoxifen or aromatase inhibitor, if necessary, according to the hormonal (positive) receptor status, were accepted for the present study.

Patients with distant metastasis reaching bones, viscera (lung and liver), central nervous system and skin (synchronic metastasis to different sites) were excluded from the present study.

Identification of metastatic sites

Metastatic sites were identified by imaging bone scintigraphy, radiography, computed tomography and nuclear magnetic resonance, when indicated.

Regarding the metastatic sites in the bones, these were subdivided into three groups: long bones, short (flat) bones and both.

The long bones considered were: femur, tibia, fibula, humerus, radius, ulna and clavicle. And among short or flat bones: bones of the skull, spine, sternum, ribs and pelvis.

The routine immunohistochemical analysis was done with the collaboration of Dr. Francisco Carlos Quevedo and Dr. Francisco Alves Moraes Neto, Department of Pathology, *Hospital Amaral Carvalho*, Jaú.

To facilitate the analysis of this work, and in view of tumor biological behavior, the molecular subtypes were grouped into four groups in Table 2.

RESULTS

The results are described in the form of tables and graphics. The statistical results are indicated with their corresponding p-value; and the tests are named when necessary.

Table 2. Groups of molecular subtypes.

Grouping	Subtypes
Subtype 1	Luminals A and B
Subtype 2	Group HER2+
Subtype 3	Hybrid luminal group
Subtype 4	Triple-negative and basal-like group

HER2: human epidermal growth factor type 2.

The histological classification of the tumors evaluated in this study is organized in Table 3.

About molecular subtypes and the detection of implants in long bones, short (flat) bones or both

As shown in Table 4, of the total of 58 cases, the tendency to be implanted in flat bones in the luminal molecular subtypes was evidenced, totaling 24 cases. In long bones, three cases were obtained, and in both types (long and flat), eight cases, totaling 35 cases.

DISCUSSION

The investigation of exclusively bone metastasis becomes difficult, since the metastasis are usually implanted simultaneously, in multiple sites[4].

The breast tumor samples from these 58 patients were classified according to type and histological degree.

Of this total, 51 cases were classified as ductal carcinomas, whose histological grade ranged from 1 to 3, being 1 well differentiated and 3 undifferentiated. The majority found was histological grade 2, that is, moderately differentiated. The other forms found were mucinous carcinoma (one case), lobular infiltrating (four cases) and apocrine carcinoma (one case), and one case without histological classification.

Table 3. Histological types and respective classification of histological grade.

Histological types	Number of cases
Infiltrating ductal carcinoma G3	15
Infiltrating ductal carcinoma G2	35
Infiltrating ductal carcinoma G1	1
Mucinous carcinoma	1
Infiltrating lobular carcinoma	4
Apocrine carcinoma	1
No classification for histological rating	1
Total cases	58

Table 4. Distribution of bone metastasis according to molecular subtypes.

Bones/Molecular subtypes	Long	Flat (short)	Both	Total
Luminal A	1	16	3	20
Luminal B	2	8	5	15
Hybrid	1	5	-	6
Triple-negative	1	5	5	11
HER2	-	4	2	6

HER2: human epidermal growth factor type 2.

These numbers are in agreement with the literature data, since ductal carcinomas represent 80% of the breast tumors, and the lobular tumors, approximately 10%. The other forms represent 1% of breast cancers, in their respective classifications[9].

In this sample of 58 cases, the immunohistochemical analysis revealed 35 cases classified as luminal molecular subtypes A and B; 6 cases, HER2+ subtype; 6 cases, hybrid luminal subtype; and 11 cases, triple-negative/basal like. These numbers were corroborated by Barros and Leite in a recent review article[8].

The analysis of the correlation between the molecular subtypes of breast carcinomas (luminal A and B, luminal hybrid, triple-negative/basal like, HER2+) and implants for flat bones, long bones or both (Table 4) observed 58 metastatic cases, 24 cases for flat bones in luminal molecular subtypes; 3 cases in long bones and 8 cases in long and flat bones, totaling 35 cases.

It is known that 60 to 70% of mammary tumors are of the luminal molecular subtype A and B[8]. In the sample, we identified 35 cases of luminal bone implants, a prevalence of 60.34%, considered high in comparison to other molecular subtypes. These data are corroborated by the finding in the literature, according to Wei et al.[2].

It should be noted that 24 of the 35 metastatic luminal cases were only for flat bones, that is, approximately 70% of the cases.

These findings, in general, can contribute to the clinical practice of oncologists, especially mastologists, in light of the fact that luminal subtypes have a preference for bone implants, with 60% corresponding to flat bones.

Thus, clinical practice is recommended for care in the first months of follow-up after surgery, especially in cases of luminal subtypes, for the request of bone scintigraphy in the search for possible bone metastasis.

New studies, especially using a larger sample, are necessary to affirm or not some relation of what was studied here.

CONCLUSIONS

Due to the heterogeneity of its clinical and histopathological presentation and the difficulty of selecting cases of metastatic breast cancer exclusively for bone, the present study met the proposed objectives and was able to conclude:

- Bone metastasis were found in long bones, flat bones or both, depending on the molecular subtypes of breast carcinoma and their possible correlations. Of the 58 cases analyzed, 38 were implanted in flat bones, thus distributed: 24 in the luminal subtypes, 5 in the hybrid, 5 in the triple negative and 4 in the HER2. As to the implant in long bones, 5 cases were identified, thus distributed: 3 in the luminal subtypes, 1 in the hybrid and 1 in the triple-negative/basal like. Regarding the occurrence of both types of bones, 15 metastatic implants were found: 8 in luminal subtypes, 5 in triple-negative/basal-like and 2 in HER2;
- The molecular subtypes of breast tumors classified as luminal (A and B), triple-negative/basal-like, HER2 group and hybrid luminal were identified by immunohistochemical reaction. It has also been observed that luminal molecular subtypes form the majority of bone metastasis.

Finally, these data also indicate the need for molecular-level research using these common molecular subtypes of breast cancer in the search for possible tumor markers for bone metastasis.

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