TC\textsuperscript{99m}M-BISPHOSPHONATES SCINTIMAMMOGRAPHY IN RELATION WITH THE POOR PROGNOSIS MARKER HER-2/NEU IN BREAST CANCER

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ABSTRACT

Objective. The uptake TC\textsuperscript{99m}M-bisphosphonates in primary breast cancer has already been proved. Statistically significant associations were found between malignant microcalcifications and HER-2/neu positivity. The aim of this study was to evaluate the correlation between TC\textsuperscript{99m}M-diphosphonates uptake and overexpression of HER-2/neu in breast cancer.

Materials and Methods: Anterior and lateral views of the breast were performed 10 min after injection of 15-20 mCi of TC\textsuperscript{99m}M-MDP or TC\textsuperscript{99m}M-PP in 7 women with suspicion of breast cancer. Breast cancer was later histologically diagnosed in all these 7 cases. Using a monoclonal antibody against HER-2/neu (Hercep test) an immunohistochemical study for the expression of HER-2/neu protein was performed in tissue sections of these 7 primary breast carcinomas.

Results: Expression of the HER-2/neu protein was detected in 3 cases, being strongly positive in 2 cases and weakly positive in 1 case. Of these patients 6 showed positive TC\textsuperscript{99m}M-MDP or TC\textsuperscript{99m}M-PP uptake (true positive) while 1 was a false negative result. Correlation analysis was used for evaluation of these results and showed an unsure correlation between scintimammography and HER-2/neu overexpression.

Conclusions: 1. There is a correlation below statistic signification between bone scanning agents uptake by breast cancer and HER-2/neu overexpression. 2. There are many uptake mechanisms, one which implies the presence of microcalcification and other associated cellular mechanisms.

Key words: scintimammography, HER-2/neu, bisphosphonates

INTRODUCTION

Primary breast cancer accumulation of bone scanning agents (TC\textsuperscript{99m}M-MDP, TC\textsuperscript{99m}M-HEDSPA, TC\textsuperscript{99m}M-PP) has been reported for a long time.\textsuperscript{1,2} More recently (1995) good results in the uptake of TC\textsuperscript{99m}M-MDP to the primary breast cancer have been reported by S. Piccolo.\textsuperscript{3} Similar results have been obtained in the Laboratory of Nuclear Medicine Timisoara, with relatively good sensitivity, specificity, positive (PPV) and negative (NPV) predictive values.\textsuperscript{4}

In trying to explain why the malign mammary nodule is depicted with breast seeking agents it is remarkable that there has been a description of microcalcifications presence in breast cancer since 1951 (Leborgne).\textsuperscript{5}

Since then, several notes of the presence of microcalcification in breast cancer have been made.\textsuperscript{6-8} On the other hand, the breast cancer cell expresses phenotypic properties which are similar to the skeletal cell type and this cell secretes bone specific proteins (osteopontine, osteonectine, bone sialoprotein).\textsuperscript{9-11}

Another overexpressed protein, HER-2/neu, which is a tyrosine kinase receptor was found to have statistically significant associations with breast cancer microcalcifications presence and with comedo histologic subtype.\textsuperscript{12, 13} Her-2/neu protein is overexpressed in 10-30% of women with breast cancer and is associated with a high level of proliferation rate and poor prognosis.\textsuperscript{14}

The aim of this study was to find out if the uptake of bone seeking agents (TC\textsuperscript{99m}M-MDP and TC\textsuperscript{99m}M-PP) in breast cancer is influenced by the presence of HER-2/neu protein overexpression in these tumors. HER-2/neu protein overexpression was evaluated immunohistochemically (using HERCEP-test kit).

MATERIALS AND METHODS

A number of 7 cases were investigated by immunohistochemistry. Biologic tissues (taken from the
archive) were used, being investigated by the Department of Histology from Medical School of Timisoara. The immunohistochemical assessment for HER-2/neu overexpression was made with HERCEP-test kit (Dako, Denmark).

Biologic tissues, taken by surgical operation, were embedded and cut at 5 µm. After that they were deparaffinized, and rehydrated to distilled water. The antigen retrieval was made with citrat, buffered at pH-6 and heating for 40 min. Then the sections were incubated with hydrogen peroxide 3% in distilled water for 5 min (in order to block endogenous peroxidase activity), then were washed with distilled water, and then with phosphate-buffered saline for 5 min. The cells were incubated with primary antibody for 30 min, then were washed with phosphat-buffered saline for 5 min and were incubated with chromagen substrate. Color was developed with DAB for 10 min. The sections were washed with distilled water, received nuclear staining with Mayer hemathoxyline, and were mounted with Canada balsam.

Tumors were further scored taking into account the frequency of immunostained malignant cells (> 10% or < 10%). The cut-off threshold used for HER-2/neu overexpression is presented in table 1. External positive control was also used . Scintimammography with Tc99m-MDP (in 6 cases) and Tc99m-PP (in 1 case) was performed during presurgical bone imaging in those 7 women. Images were acquired 10 min after the injection of 15-20 mCi Te99m-MDP or PP. Static acquisitions of each breast was performed using a low-energy collimator. Breast scintigraphy was performed with the patient lying in the prone position on a foam cushion (35 cm high), overlying the imaging table with a cut out (30/25/25 cm) for the breast. A lateral image was then performed of the dependent breast. After that an anterior image was performed for each patient. Interpretation of scintimammography was graded on a 4 point scale (Table 2).

<table>
<thead>
<tr>
<th>Uptake pattern</th>
<th>Type</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>equal to soft tissue</td>
<td>0</td>
<td>normal</td>
</tr>
<tr>
<td>diffuse or patchy uptake</td>
<td>1</td>
<td>normal/benign</td>
</tr>
<tr>
<td>without net focalization</td>
<td>2</td>
<td>malignant nodule</td>
</tr>
<tr>
<td>low -moderate intensity,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diameter &lt;10 mm</td>
<td>3</td>
<td>malignant nodule</td>
</tr>
<tr>
<td>moderate-high intensity,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diameter &gt;10 mm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Histopathology, scintimammographic results, and HER-2 /neu levels are presented in table 3.

The results of scintimammography were correlated with the immunohistochemical results for HER-2/neu protein (y = 2.92 -0.407) ( Fig 1). Correlation coefficient r is -0.61 (no statistical significance P> 0.05). The point with x=1, y=2 represent patients 2 and 5. Two illustrative cases are shown in figures 2, 3.

As shown in table 3, overexpression of HER-2/neu protein was detected in 3 cases, being strongly positive in 2 cases and weakly positive in 1 case. Of these patients, 6 showed positive Te99m-MDP or Te99m-PP uptake and 1 was a false negative result (1 out of 7 cases failed to be depicted -14.3%).

DISCUSSIONS

A high affinity for hydroxyapatite (together with other mechanisms) enables radiolabeled bisphosphonates to be used in bone scanning. An explanation for primary breast cancer uptake of these radiopharmaceuticals (bisphosphonates and PP) could be the presence of hydroxiapatite at the microcalcification level while it is known that bisphosphonates and pyrophosphate strongly bind to the hydroxiapatite in bone. There are no reports in the literature regarding the correlation between bone specific proteins (osteopontin, osteonectin, bone sialoproteine) which are proved to be specific for breast cancer tumors and HER-2/neu overexpression.

HER-2/neu protein overexpression uses several signaling pathways, such as the ras/mitogen-activated protein kinase pathway that is important for mitogenic stimulation and kinase cascade. HER-2/neu is associated with comedo histologic subtype and microcalcifications.

This group of patients is an initial group, studied for the sole purpose of finding out if this study is justified. Our results (correlation coefficient r = -0.61, P>0.05 ) show an unsure correlation between bone...
seeking agents uptake by the breast cancer and this protein (Her-2/neu).

Therefore, it is possible that HER-2/neu protein overexpression influences in a minor way the visualisation of breast cancer with bone seeking agents. Also, the low statistic positivity of this marker (10-30% of human breast cancer) is discordant with relatively good results on scintimammography. There are no reports in the literature of the investigation of the correlation between uptake of bone seeking agents in the breast cancer and HER-2/neu protein overexpression.

It is possible that the binding of microcalcification is not the only uptake mechanism of these agents and that there are other cellular mechanisms involved as well. One of them could be the proved action mechanism of nonnitrogen bisphosphonates in bone metabolism, where these bisphosphonates are incorporated into ATP-containing compounds so that they become nonhydrolizable and inhibit cell function and may lead to cell apoptosis making the imaging with radiolabeled bisphosphonates possible.

For HEDSPA, a bisphosphonate which was used in the past, and by us too for SMG, with good results, it was proved to inhibit some protein-tyrosine phosphatases (changing the phosphate metabolism). This interaction with protein-tyrosine phosphatases may have a role in the uptake of these agents in the breast cancer.

![Image of uptake vs HER-2/neu graph]

**Figure 1.** Scintimammographic results correlated with HER-2/neu expression

<table>
<thead>
<tr>
<th>No</th>
<th>Age</th>
<th>Size</th>
<th>Histology</th>
<th>Radiopharmaceutical</th>
<th>Scintimammographic findings</th>
<th>HER-2/neu</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>41</td>
<td>2 cm</td>
<td>Infiltrating ductal carcinoma, comedo subtype, G2</td>
<td>MDP</td>
<td>+1</td>
<td>+3</td>
</tr>
<tr>
<td>2</td>
<td>69</td>
<td>4 cm</td>
<td>Infiltrating ductal carcinoma, G2</td>
<td>MDP</td>
<td>+2</td>
<td>+1</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>3 cm</td>
<td>Infiltrating ductal carcinoma, G2</td>
<td>MDP</td>
<td>+3</td>
<td>+1</td>
</tr>
<tr>
<td>4</td>
<td>81</td>
<td>4 cm</td>
<td>Infiltrating ductal carcinoma, G2</td>
<td>MDP</td>
<td>+3</td>
<td>+2</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>2 cm</td>
<td>Infiltrating ductal carcinoma, G2</td>
<td>MDP</td>
<td>+2</td>
<td>+1</td>
</tr>
<tr>
<td>6</td>
<td>66</td>
<td>2 cm</td>
<td>Infiltrating ductal carcinoma, G2</td>
<td>MDP</td>
<td>+2</td>
<td>+3</td>
</tr>
<tr>
<td>7</td>
<td>56</td>
<td>2 cm</td>
<td>Infiltrating ductal carcinoma, G2</td>
<td>PP</td>
<td>+3</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table 3.** Scintimammographic results correlated with histopathology and HER-2/neu levels

**Figure 2.** Patient 1 from table III with uptake +1 and HER-2/neu +3

**Figure 3.** Patient 3 from table III with uptake +3 and HER-2/neu +1
CONCLUSIONS

1. There is a correlation below statistic significance between bone scanning agents uptake to the breast cancer and HER-2/neu overexpression.
2. There are many uptake mechanisms, one which imply microcalcification presence and other associated cellular mechanisms.

REFERENCES