Prognostic Impact of Marginal Adipose Tissue Invasion in Ductal Carcinoma of the Breast

Junzo Yamaguchi, MD, Hiroshi Ohtani, MD, Kazukuni Nakamura, MD, Isao Shimokawa, MD, and Takashi Kanematsu, MD

Key Words: Breast cancer; Adipose tissue invasion; Lymph node status; Prognosis; Lymphatic vessel invasion

Abstract

This study aimed to investigate whether adipose tissue invasion (ATI) of cancer cells at the tumor margin influenced lymph node status and prognosis in patients with invasive ductal carcinoma of the breast. Data for 245 patients with breast cancer with marginal ATI were clinicopathologically compared with data for 65 patients without ATI. We also examined the value of the combination of ATI and peritumoral lymphatic vessel invasion (LVI). The frequency of axillary lymph node metastases was 40.7% in patients with ATI (99/243) and 11.3% in patients without ATI (7/62; P < .0001), and ATI was an independent factor influencing nodal metastasis. Patients with ATI had a poorer prognosis than patients without ATI (10-year disease-free survival, 76% and 94%, respectively; P = .0323). In addition, patients without ATI or LVI had neither lymph node metastasis (n = 52) nor recurrent disease (n = 53). ATI is one of the biologic indicators of tumor aggressiveness.

The spread of cancer cells via the lymphatic system is the first step in the dissemination of breast cancer, but little is known as to how cancer cells enter the lymphatic vessels. It has been speculated that lymph node metastasis is promoted by tumor cell aggressiveness, ie, invasiveness, motility, and proliferation. Tumor-stromal interactions seem to be a fundamental aspect of tumor aggressiveness. It has also been suggested that functional lymphatics at the tumor margins are responsible for lymphatic metastasis. Therefore, we propose that the ability to invade neighboring tissues is more significant than the ability to proliferate with regard to the tumorigenic-metastatic potential of breast cancer cells. In contrast, these tumor characteristics indicate the need to identify reliable markers to predict patients at low risk who do not require adjuvant chemotherapy or endocrinotherapy.

The International Union Against Cancer classification has shown the significance of chest wall and skin invasions in evaluating local tumor extension in breast cancer. Some investigators noted that pathologic evidence of fat invasion, such as scattered invasion into fat tissues and the invasive length of fat invasion, was related to a poor prognosis. However, the prognostic significance of adipose tissue invasion (ATI) at the tumor margin has not been fully evaluated in breast cancer, and the biologic characteristics of tumors with ATI are also insufficiently known. Meanwhile, many studies have been conducted to define the significance of lymphovascular invasion. Peritumoral lymphatic vessel invasion (LVI) is associated with a higher frequency of nodal metastasis and has also been cited as a risk factor in breast carcinoma.

Therefore, we hypothesize that the combination of the values of marginal ATI and peritumoral LVI may be an important tool for risk allocation in patients with breast cancer because
the invasive potential of cancer cells may be critical for the early dissemination of breast cancer. We looked at marginal ATI in invasive ductal carcinoma of the breast and investigated the relationship between ATI and other clinicopathologic findings to clarify the biologic behavior of breast cancer with ATI. Finally, we evaluated the prognostic value of the combination of marginal ATI and peritumoral LVI to confirm our hypothesis.

Materials and Methods

In the present study, we defined ATI with strict emphasis on reproducibility and simplicity and restricted the cases to early stage breast carcinoma to assess the biologic characteristics of marginal ATI in invasive ductal carcinoma.

Patients

A retrospective analysis was conducted using the data for 310 patients with invasive ductal carcinoma of the breast who had undergone surgery (total or partial mastectomy) from 1993 to 2004. The study materials represented 58.6% of all new breast carcinomas diagnosed in the same period (310/529). Except for 5 cases, the patients had all undergone axillary lymph node dissection or a sampling procedure. Data for patients with a special histologic type such as mucinous, lobular, medullary, or squamous cell carcinoma were excluded from the study. We also excluded data for patients with bilateral breast cancer (synchronous, metachronous), clinically multifocal or multicentric tumors in the unilateral breast, skin or striated muscle invasion, inflammatory carcinoma, distant metastasis, or malignancy at another site and for patients who had received preoperative neoadjuvant chemotherapy.

The patient age ranged from 27 to 87 years (median, 52 years). Body mass index (BMI) was calculated (kg/m²) in all patients. In the study, 300 (96.8%) of 310 were followed up until October 31, 2006; the median follow-up period was 63 months (range, 7-164 months).

Tissue Preparation and Tumor Size

The resected breast and lymph node tissues were fixed in 10% neutral buffered formalin, and the breast tissues were cut into 5-mm slices. Each paraffin-embedded block was cut into 4-µm sections and stained with H&E. All but a few specimens from surgery, excision biopsy, and mammatome (vacuum-assisted) biopsy were available for histologic examination. Tumor size was measured as the largest dimension of the microscopic invasive component on the pathologic specimen (range, 0.1-4.0 cm, pT1-2). Lymph node involvement was pathologically assessed in 305 patients who underwent axillary dissection. The numbers of patients with disease at each pTNM0 stage were as follows: pT1N0, 171; pT2N0, 28; pT1N+, 67; pT2N+, 38; and unknown, 6.

Definitions of Adipose Tissue and Marginal Adipose Tissue Invasions

In the present study, adipose tissue was defined as a pure aggregate consisting of more than 20 fat cells without intervening fibrous tissues in the breast. The adipose tissue included tissues surrounding the mammary ducts or lobules and those in the subcutaneous layers. Fibroadipose tissue (fat cells mixed with various fibrous tissues) was strictly distinguished from adipose tissues (Image 1A). Marginal ATI was defined as the presence of more
than 20 cancer cells in direct contact with the adipose tissue or the location of cancer cells in the adipose tissue. Only cases with unequivocal ATI were considered positive (ATI+). Doubtful cases were considered negative (ATI−).

Peritumoral LVI

LVI was defined as the presence of peritumoral lymphatic emboli in endothelium-lined spaces, according to the literature. Immunohistochemical analysis using lymphatic endothelial markers such as D2-40 was not performed. Samples with obvious emboli were considered positive (LVI+), and doubtful samples were considered negative (LVI−).

Histologic Grade and Hormone Receptor Status

All tumors were graded according to a modified version of the Bloom-Richardson histologic grading criteria. The status of estrogen and progesterone receptors was evaluated by immunohistochemical analysis using commercially available antibodies (DakoCytomation, Kyoto, Japan). Cases with more than 10% of the tumor cell nuclei stained were defined as positive.

Statistics

The χ² analysis and Student t test were used to analyze the differences between study groups. A multivariate analysis was conducted to investigate the factors influencing nodal involvement or marginal ATI. Survival curves for cancer-specific survival were constructed using the method of Kaplan and Meier, and the differences between the 2 groups were assessed by using the Breslow-Gehan-Wilcoxon test. Overall survival was calculated as the period from surgery to death of breast cancer. Probability (P) values were calculated using StatView 5 software (SAS Institute, Cary, NC). P values less than .05 were considered statistically significant. Information on tumor size, lymph node status, hormone receptor status, or survival was unavailable for some patients.

Results

Relationship Between ATI and Clinicopathologic Parameters

Of 310 cases, 245 (79.0%) showed marginal ATI. As shown in Table 1, ATI+ cases had the highest frequency of axillary lymph node metastases (99/243 [40.7%]) compared with the ATI− cases (7/62 [11.3%]) (P < .0001). Patient age (P = .0009) and pathologic tumor size (P < .0001) were significantly related to ATI. No statistical differences in BMI, LVI, histologic grade, or hormone receptor status (estrogen receptor and progesterone receptor) were observed. ATI was an independently significant factor influencing nodal involvement according to multivariate analysis (P = .0079).

Table 1. Because patient age and pathologic tumor size were closely related to ATI, these 2 parameters were used for multivariate analysis. Age (P = .0012) and tumor size (P < .0001) were independently significant factors influencing marginal ATI (data not shown).

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ATI+ (n = 245)</th>
<th>ATI− (n = 65)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td>&lt;.0001*</td>
</tr>
<tr>
<td>Present</td>
<td>99</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>144</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD age (y)</td>
<td>55.4 ± 11.9</td>
<td>49.9 ± 11.1</td>
<td>.0009†</td>
</tr>
<tr>
<td>Mean ± SD body mass index (kg/m²)</td>
<td>23.3 ± 3.3</td>
<td>22.5 ± 3.9</td>
<td>.0627†</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>1.7 ± 0.6</td>
<td>1.0 ± 0.7</td>
<td>&lt;.0001†</td>
</tr>
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<td>Lymphatic vessel invasion</td>
<td></td>
<td></td>
<td>.0501*</td>
</tr>
<tr>
<td>Positive</td>
<td>71</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>174</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Histologic grade</td>
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<tr>
<td>I</td>
<td>79</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>106</td>
<td>18</td>
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<tr>
<td>III</td>
<td>60</td>
<td>23</td>
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</tr>
<tr>
<td>Estrogen receptor</td>
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<td>.0689*</td>
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<tr>
<td>Positive</td>
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<td>33</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>77</td>
<td>28</td>
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<tr>
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<td></td>
<td>.1858*</td>
</tr>
<tr>
<td>Positive</td>
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<td>28</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>98</td>
<td>32</td>
<td></td>
</tr>
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</table>

ATI, adipose tissue invasion.

* χ² analysis.
† Student t test.
Prognostic Value of ATI

Data for 237 ATI+ cases and 64 ATI– cases were available for the survival analysis. The 5- and 10-year disease-free survivals were 83% and 76%, respectively, in ATI+ cases, and both values were 94% in ATI– cases [Figure 1A] (P = .0323). The 10-year overall survivals were 81% and 98% in ATI+ and ATI– cases, respectively [Figure 1B] (P = .0577).

Prognostic Value of LVI

Data for 80 LVI+ cases and 220 LVI– cases were available for the survival analysis. The 5- and 10-year disease-free survivals were 69% and 60%, respectively, in LVI+ cases and were 92% and 90%, respectively, in LVI– cases [Figure 2A] (P < .0001). The 10-year overall survivals were 66% and 91% in LVI+ and LVI– cases, respectively [Figure 2B] (P = .0008).

Significance of the Combination of ATI and LVI

In the next step, we investigated the clinical relevance of marginal ATI when combined with peritumoral LVI. Lymph node metastasis was found in 75% of ATI+/LVI+ cases (53/71) and 70% of ATI–/LVI+ cases (7/10). In ATI+/LVI– cases, the frequency was 26.7% (46/172). However, no nodal metastasis was observed in ATI–/LVI– cases (0/52).

Table 2

Multivariate Analysis for Nodal Involvement in 304 Cases of Ductal Carcinoma of the Breast

<table>
<thead>
<tr>
<th>Factor</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphatic vessel invasion</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Tumor size</td>
<td>.0004</td>
</tr>
<tr>
<td>Adipose tissue invasion</td>
<td>.0079</td>
</tr>
</tbody>
</table>

Figure 1

Disease-free (A) and overall (B) survival for 237 adipose tissue invasion (ATI)+ and 64 ATI– cases of ductal carcinoma of the breast. The 5- and 10-year survivals (%) are shown. A, P = .0323; B, P = .0577; Wilcoxon test for both.

Figure 2

Disease-free (A) and overall (B) survival for 80 lymphatic vessel invasion (LVI)+ and 220 LVI– cases of ductal carcinoma of the breast. The 5- and 10-year survivals (%) are shown. A, P < .0001; B, P = .0008; Wilcoxon test for both.
Moreover, as shown in Figure 3I, the 5- and 10-year disease-free survivals were 69% and 59%, respectively, in ATI+/LVI+ cases (n = 70); 89% and 85%, respectively, in ATI+/LVI− cases (n = 167); and 100% in ATI−/LVI− cases (n = 53). Data for the ATI−/LVI+ cases (n = 11) were excluded from Figure 3. On the other hand, no difference was observed in disease-free survival between the LVI+ (Figure 2A) and ATI+/LVI+ cases (Figure 3).

**Discussion**

The present study revealed 3 novel findings. First, ATI of cancer cells at the tumor margin was independently associated with nodal involvement in patients with invasive ductal carcinoma of the breast. Second, patient age and invasive tumor size were significant factors for ATI. Finally, patients without ATI or LVI had an excellent prognosis.

The breast stroma histologically consists of fibrous tissues and adipocytes in variable proportions, and the adipocytes physiologically increase with aging in older women. Generally, when intraductal carcinoma cells infiltrate the breast stroma, the cells initially penetrate the fibrous tissues, followed by the fibroadipose tissues, and, finally, the adipose tissues in breast cancer (Image 1). We focused on the ATI of cancer cells and established strict criteria for adipose tissue and ATI in histologic examinations.

Marginal ATI of cancer cells correlated with lymph node metastasis. Although the relationship between ATI and LVI was statistically insignificant (P = 0.501; Table 1), marginal ATI may lead to a larger contact area between cancer cells and the peritumoral functional lymphatic endothelium. The presence of ATI may reflect the infiltrating growth pattern of cancer cells at the marginal site, while the absence of ATI may be associated with an expanding growth pattern or the existence of abundant fibrous tissues surrounding the ducts and lobules as a result of other underlying conditions such as fibrocystic changes and fibrous mastopathy. Furthermore, stromal reaction patterns, including edema and desmoplasia, in invasive cancer may affect ATI or prognosis in patients with breast cancer.18

In most patients with breast cancer, only fibrous or fibroadipose tissue invasion by cancer cells appears insufficient for nodal metastasis. The true mechanisms of ATI-associated nodal metastasis are unknown, but distinct molecular mechanisms are likely to be concerned with ATI- and peritumoral LVI-associated nodal metastasis. Aromatase activity and adipocytokines such as leptin in mammary adipose tissues may participate in ATI-associated nodal involvement. Several investigators have reported that aromatase activity and its expression in breast adipose tissues are most prominent in regions proximal to the tumor in breast cancer.19-21

There is a possibility that tissue estrogen concentration is higher in breast carcinoma with ATI. This may cause the aggressive biologic behavior of breast cancer with ATI and the subsequent involvement of the lymphatic network.22 In addition, mammary adipose tissue is an important source of various adipocytokines, including leptin. Leptin is one of the neurohormone regulators in the hypothalamus and is necessary to normal mammary gland development and lactation. It might be also involved in carcinogenesis and progression in breast cancers.23 Such paracrine adipocytokines may contribute to tumor aggressiveness and ATI-associated nodal metastasis.

Alkarain et al24 recently reported that the lymphatic endothelial marker D2-40 is useful for detecting intratumoral obliterated LVI that cannot be visualized on H&E-stained sections. These intratumoral LVis in adipose tissue may be associated with nodal metastasis. Taken together, further investigations on the molecular mechanisms of vessel invasion and genetic participation in the invasive process are essential for anticancer therapy, including new molecular target therapy.

The size of the invasive component, a significant factor for ATI in the present study, has been shown to be closely related to nodal involvement.25 In the current setting, not only ATI but also tumor size (P = .0004) were shown to be independently significant factors influencing nodal metastasis (Table 2). It is natural to assume that enlarged tumors can extend into adipose tissues and acquire greater opportunities for cancer cell intravasation at the tumor margin.

Moreover, in the present study, patients with ATI+ disease were significantly older than patients with ATI− disease.
Because the BMI showed no significant association with ATI, ATI may be affected by an age-related increase in fat cells (fatty change) in the breast stroma rather than the quantity of body adipose tissue. However, age was not a prognostic factor in this study. Patients with LVI+ disease were significantly younger than patients with LVI− disease (P < .0001), being in sharp contrast to the relationship between ATI and patient age. We think that breast tissue is less fatty and cancer cells are more aggressive in younger patients, whereas breast tissue is more fatty and cancer cells are less aggressive in older patients.

Peritumoral lymphovascular invasion is thought to be a useful factor for risk allocation,12,15,16 and at the St Gallen meeting in 2005, lymphovascular invasion was newly added to the list of adverse prognostic factors in patients with node-negative disease.14 Schoppmann et al12 have shown that the 10-year disease-free survival rates were 45% and 70% in patients with (n = 105) and without (n = 269) lymphovascular invasion, respectively. They also indicated the predictive value of lymphovascular invasion in the development of lymph node metastasis. These observations are more or less consistent with our findings. In the present study, no prognostic significance of ATI could be found in LVI+ cases, but it was particularly interesting that patients without ATI or LVI had neither nodal involvement nor recurrent disease. These patients are expected to be free of distant metastasis. The combination of these 2 microscopic features may be a useful predictive factor in identifying patients who require no axillary dissection, chemotherapy, or hormonal treatment.

From this series of investigations, ATI of cancer cells at the tumor margin is one of the biologic indicators of tumor aggressiveness in early stage breast cancer. We came to the conclusion that ATI+ cases were associated with adverse outcomes in addition to the converse, that ATI− cases were associated with favorable outcomes. We think that ATI should be incorporated in a standard surgical pathology report of infiltrating ductal carcinoma of the breast. The precise evaluation of marginal ATI and peritumoral LVI will prove to be useful in the formulation of therapeutic strategies and the prediction of which patients with breast cancer have an excellent prognosis.

References


From the 1Department of Surgery, National Hospital Organization, Saga Hospital, and 2Department of Investigative Pathology and Surgery II, Nagasaki University Graduate School of Biomedical Science, Nagasaki, Japan.

Address reprint requests to Dr Yamaguchi: Dept of Surgery, National Hospital Organization, Saga Hospital, 1-20-1 Hinode, Saga 849-0923, Japan.


