Original Article

Invasive cribriform carcinoma of the breast: a clinicopathological analysis of 12 cases with review of literature

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Abstract: Invasive cribriform carcinoma (ICC) is a rare type of invasive breast cancer. We aim to investigate the clinicopathological features, immunophenotypes, diagnosis and differential diagnosis of ICC. Thus, clinicopathological data of 12 ICC patients were collected. All 12 cases were female, aged 38 to 75 years, with a median age of 53 years old. The maximum diameter of the tumor was 2 cm to 10 cm, in which the median tumor size was 2.54 cm in pure ICC and classical ICC. Microscopically, the cancer nests of ICC assumed an invasive, irregular island-shaped distribution, with an irregular mesh structure internally and fibrous reactions around most cancer nests. 67% (8/12) of cases were grade 1 and 33% (4/12) of cases were grade 2 tumors. Immunohistochemically, ER and PR were moderately to strongly positive with the positive tumor cell number accounting for 30% to 95% in all cases. HER-2 was negative in all cases except in one case which was positive (2+). Myoepithelial markers such as Calponin, p63, CK5/6 and CD10 were all negative in the cancer nests. 58% (7/12) of cases had a ki67 index of ≤ 14%. All follow-up patients were followed for 12 to 70 months (with a mean of 42 months), and were disease-free after treatment except for one patient whom we lost during the follow up. In conclusion, ICC, as a special type of breast cancer, has its unique clinicopathological and immunophenotypic characteristics, leading to a good prognosis.

Keywords: Invasive cribriform carcinoma (ICC), invasive ductal carcinoma (IDC), immunohistochemistry, breast

Introduction

Invasive cribriform carcinoma (ICC) is a rare type of invasive breast cancer with an incidence of approximately 0.3%-6% in primary breast carcinomas [1-4], and having a cribriform pattern resembling the histological structures of cribriform ductal carcinoma in situ (cribriform DCIS) [5, 6]. ICC is considered a malignant neoplasm with a low metastatic potential and a good prognosis [1, 2, 5]. ICC is generally divided into pure, classical, and mixed forms [2, 5]. Pure ICC consists of an invasive cribriform pattern in > 90% of the lesions. Classical ICC is described as exhibiting a predominant invasive cribriform pattern, accompanied by < 50% of a tubular carcinoma (TC). If accompanied by 10%-49% of other invasive carcinoma components (except tubular carcinoma), it should be classified as a mixed type. ICC occurs rarely and is usually associated with smaller tumors along with fewer lymph nodes metastasis and lower tumor stages, providing a better prognosis compared to ordinary breast cancers [2, 5, 7]. However, the prognostic significance of the clinicopathological characteristics of ICC patients is not clearly established due to its low incidence and its lack of a standard definition. In addition, distant metastasis of ICC has rarely been found till now, with only individual reports that can be seen [7]. Most patients with ICC express a positive estrogen receptor (ER) and progesterone receptor (PR) [8], while the amplification of the human epidermal growth factor receptor 2 (HER-2) is rarely detected, consequently, classifying ICC as a luminal breast cancer [4, 9]. At present, the study of ICC is mostly based on case reports or small retrospective studies due to its low incidence. Hence, in this study, in order to investi-
## Table 1. Clinicopathological features of 12 cases of ICC

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Years)</th>
<th>Menopause</th>
<th>Laterality</th>
<th>Tumor Max Diameter (cm)</th>
<th>Surgery</th>
<th>Tumor grade</th>
<th>Lymph Node Metastasis</th>
<th>Type</th>
<th>TNM Staging</th>
<th>ER, PR, HER-2 status</th>
<th>Chemotherapy</th>
<th>Follow-up time (Months)</th>
<th>Proliferative fraction (Ki67)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>Yes</td>
<td>Left</td>
<td>2.4</td>
<td>BCS+SLNB</td>
<td>1</td>
<td>0/3</td>
<td>Pure</td>
<td>IIA</td>
<td>ER (80%+, MP) PR (90%+, SP), HER-2 (0)</td>
<td>No</td>
<td>12</td>
<td>10%</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>No</td>
<td>Left</td>
<td>2.5</td>
<td>BCS+SLNB</td>
<td>1</td>
<td>0/2</td>
<td>Pure</td>
<td>IIA</td>
<td>ER (30%+, MP) PR (60%+, SP), HER-2 (0)</td>
<td>No</td>
<td>12</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>Yes</td>
<td>Left</td>
<td>3.0</td>
<td>M</td>
<td>2</td>
<td>No lymph node examination</td>
<td>Mixed (IDC + ICC)</td>
<td>IIB</td>
<td>ER (90%+, SP) PR (95%+, SP), HER-2 (2+)</td>
<td>Yes</td>
<td>14</td>
<td>20%</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>Yes</td>
<td>Left</td>
<td>2.0</td>
<td>M</td>
<td>1</td>
<td>0/3</td>
<td>Pure</td>
<td>IIA</td>
<td>ER (70%+, MP) PR (90%+, SP), HER-2 (0)</td>
<td>Yes</td>
<td>24</td>
<td>10%</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>Yes</td>
<td>Left</td>
<td>3.0</td>
<td>M+ALND</td>
<td>2</td>
<td>3/11</td>
<td>Pure</td>
<td>IIA</td>
<td>ER (90%+, SP) PR (70%+, MP), HER-2 (0)</td>
<td>Yes</td>
<td>37</td>
<td>7%</td>
</tr>
<tr>
<td>6</td>
<td>71</td>
<td>Yes</td>
<td>Left</td>
<td>2.5</td>
<td>M+ALND</td>
<td>1</td>
<td>0/6</td>
<td>Classical</td>
<td>IIA</td>
<td>ER (90%+, SP) PR (90%+, SP), HER-2 (0)</td>
<td>No</td>
<td>49</td>
<td>20%</td>
</tr>
<tr>
<td>7</td>
<td>62</td>
<td>Yes</td>
<td>Right</td>
<td>3.0</td>
<td>M+ALND</td>
<td>1</td>
<td>0/10</td>
<td>Pure</td>
<td>IIA</td>
<td>ER (80%+, MP) PR (90%+, SP), HER-2 (0)</td>
<td>Yes</td>
<td>56</td>
<td>20%</td>
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<tr>
<td>8</td>
<td>47</td>
<td>Yes</td>
<td>Right</td>
<td>1.2</td>
<td>M+ALND</td>
<td>1</td>
<td>0/26</td>
<td>Mixed (ICC + Mucinous Carcinoma)</td>
<td>IA</td>
<td>ER (80%+, MP) PR (70%+, MP), HER-2 (0)</td>
<td>Yes</td>
<td>63</td>
<td>20%</td>
</tr>
<tr>
<td>9</td>
<td>75</td>
<td>Yes</td>
<td>Left</td>
<td>3.0</td>
<td>M+ALND</td>
<td>2</td>
<td>2/10</td>
<td>Pure</td>
<td>IIB</td>
<td>ER (80%+, MP) PR (60%+, MP), HER-2 (0)</td>
<td>Yes</td>
<td>65</td>
<td>2%</td>
</tr>
<tr>
<td>10</td>
<td>45</td>
<td>Yes</td>
<td>Right</td>
<td>2.0</td>
<td>M+ALND</td>
<td>1</td>
<td>0/16</td>
<td>Pure</td>
<td>IA</td>
<td>ER (90%+, MP) PR (70%+, SP), HER-2 (0)</td>
<td>Yes</td>
<td>Lost</td>
<td>2%</td>
</tr>
<tr>
<td>11</td>
<td>56</td>
<td>Yes</td>
<td>Right</td>
<td>10</td>
<td>M+ALND</td>
<td>2</td>
<td>1/3</td>
<td>Mixed (IDC + ICC)</td>
<td>IIIA</td>
<td>ER (70%+, SP) PR (80%+, MP), HER-2 (0)</td>
<td>Yes</td>
<td>65</td>
<td>10%</td>
</tr>
<tr>
<td>12</td>
<td>39</td>
<td>No</td>
<td>Left</td>
<td>2.5</td>
<td>M+ALND</td>
<td>1</td>
<td>0/15</td>
<td>Pure</td>
<td>IIIA</td>
<td>ER (80%+, MP) PR (80%+, SP), HER-2 (0)</td>
<td>No</td>
<td>70</td>
<td>15%</td>
</tr>
</tbody>
</table>

Abbreviations: IDC, Invasive ductal carcinoma; DCIS, Ductal carcinoma in situ; BCS, breast-conserving surgery; M, mastectomy; SLNB, sentinel lymph node biopsy; ALND, axillary lymph node dissection. MP, moderately positive; SP, strongly positive.
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gate the clinicopathological features, immunological, diagnosis and differential diagnosis of ICC and enhance awareness of the disease, we retrospectively reviewed the clinical, histological and immunohistochemical features of 12 cases of ICC.

Materials and methods

According to the latest diagnostic criteria of ICC by the WHO’s (2012) breast tumor classification [5], 12 cases of invasive breast cancer were collected from the department of Pathology in the First Affiliated Hospital of Guangxi Medical University, the People’s Republic of China during the period of November 2011 to May 2017. The clinicopathological data was obtained from clinicians, who searched for the patient’s medical records where they were available, and included the age at diagnosis, menopausal status, laterality, tumor max diameter, tumor grade, lymph node status, hormone receptor status, HER-2 status, proliferative fraction (Ki67), as well as the treatment and outcome. The pathological tumor stage (TNM stage) was assessed according to the criteria established by the 7th edition of the American Joint Committee on Cancer (AJCC) staging manual. If information about the follow-up was not available in the medical records, we then followed the patient by telephone. The resection specimens were fixed in 10% neutral formalin, followed by conventional dehydration and paraffin embedding. The thickness of each specimen was 4 µM. Hematoxylin and eosin (H&E) staining and immunohistochemistry were performed respectively. The antibodies used include ER, PR, HER-2, Ki-67, Calponin, p63, CK5/6, CD10, E-cadherin, p120, p53, CD56, CgA, Syn. All antibodies were purchased from Fuzhou’s Maixin Company and all the experiments were performed according to EnVision’s two steps method according to the indications of the manufacturer. Each case was compared with the positive and negative external control, and if appropriate, we used a good internal control for comparison. Immunohistochemical results were identified by two pathologists in double-blind. For ER and PR, the position of the immunoreactivity, the percentage and the intensity of the stained cells were identified. A positive ER or PR was defined as having a positive staining of the tumor cells greater than or equal to 1%, and showing varying degrees of nuclear staining. A positive HER2 was defined as having more than 30% of invasive cancer cells with a uniform, intense and complete cell membrane coloring (called 3+); a negative result had an IHC staining of 0 or 1+; and an unclear result had a cell membrane coloring of moderate intensity (called 2+).

Results

Clinical features

The clinicopathological data of 12 cases of ICC are summarized in Table 1.

The 12 cases of ICC were all female, aged 38 to 75 years with a median age of 53 years. In addition, 2 of the cases were premenopausal and the other 10 cases were postmenopausal. 66% (8/12) of cases had lesions located in the left breast, 33% (4/12) of cases had lesions located in the right breast, and only 1 case was multifocal. 67% (8/12) of cases were grade 1 and 33% (4/12) of cases were grade 2 tumors. All patients accepted surgery. During surgery, 8 cases underwent modified radical mastectomy including axillary lymphadenectomy and 2 cases underwent mastectomy. Another 2 cases underwent breast-conserving surgery and sentinel lymph node biopsy. Axillary lymph node metastasis was noted in 3 cases (25%). No occupying lesions or metastatic cancer was found in additional organs. Eight of the 12 patients underwent postoperative chemotherapy, while no further adjuvant therapy was performed in the other patients. All patients, except for 1 case which was lost during the follow up, were followed for 12 to 70 months (with a mean of 42 months), and the remaining 11 patients were tumor-free after treatment.

Gross and microscopic features

The surface of the tumor was solid grey-white, hard, and the boundary was unclear with no capsule surrounding it. The maximum diameter of the tumor was 2 cm to 10 cm, in which the median tumor size was 2.54 cm in pure ICC and classical ICC, while 5.0 cm in mixed ICC. Microscopically, according to the WHO’s (2012) standard classification of invasive breast cancer [5], pure ICC consist of an invasive cribriform pattern of > 90% of the lesion. If the tumor has an invasive and a cribriform pattern, and is associated with less than 50% of a tubular...
Clinicopathological characteristics of ICC of breast

component, then it should be classified as a classical ICC. However, if the tumor has an invasive pattern and a cribriform pattern, but is accompanied with 10% to 49% of other morphological components (excluding tubular carcinoma), then it should be classified as mixed ICC. According to this standard analysis, 8 (67%) cases were classified as pure ICC, 3 (25%) cases were classified as mixed ICC and 1 case was classified as classical ICC. Among the mixed ICC group, 2 cases were comprised of invasive ductal carcinoma, and 1 case was composed of mucinous adenocarcinoma. Upon microscopic examination, invasive carcinoma showed a cribriform pattern, with an irregular island-shaped distribution and the nest having an irregular mesh structure internally (Figure 1A). 67% (8/12) of cases were grade 1 and 33% (4/12) of cases were grade 2 tumors. Reddish exudates were seen within the mesh (Figure 1B). Luminal-microcalcifications were noted in 2 of the 12 cases (Figure 1C, 1D). All tumor cells were well differentiated, with the cells being smaller, more uniform and having a round or oval nucleus with mild to moderate nuclear atypia. Mitosis was rarely observed (range: 1-3 mitoses per 10 fields at ×400 magnification). The cytoplasm of the tumor cells had visible apocrine (Figure 1E, 1F). A small number of osteoclastic-like giant cells were also noted in 1 case. All tumors had few necrotic components or none at all, and fibrous reactions were evident around most cancer nests. However, there was no obvious myoepithelial layer around the cancer nest. Also, no lymphovascular or perineural invasion was identified. The tumors retained their mesh structure during the lymph node metastasis.

In all 12 cases of ICC, ER and PR were moderately to strongly positive (with immunohistochemistry the positive tumor cell number accounts for 30% to 95%) (Figure 2A, 2B). HER-2 expression was negative, except for one case in which HER-2 expression was positive (2+). P120 and E-cadherin were positive in the cell membrane (Figure 2C-F). Myoepithelial markers, such as Calponin, p63, CK5/6 and CD10 were negative around the cancer nests (Figure 2G-I). The proliferation index of Ki-67 were in the range of 2% to 20%, among which 58% (7/12) of the cases had a ki67 of ≤ 14%, and 42% (5/12) of cases had a Ki67 > 14%. CD56, CgA and Syn were tested in 3 cases, and all of them were negative.

Discussion

ICC is more common in older women, especially postmenopausal women [6, 10], with only a few
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In this study, we retrospectively investigated the clinicopathological characteristics of 12 cases of ICC. All 12 cases of ICC were female with the average age of onset at 54 years, and the onset of disease was postmenopausal for 10 out of the 12 cases (83%). Clinically, patients usually seek medical advice after discovering a lump, but it may tend to be frequently asymptomatic. Compared to IDC, ICC presented a relatively small tumor, and most of the tumors (58%-75.4%) were less than 2 cm in diameter, and about 22%-38% of cases’ tumor size was 2-5 cm [4, 6]. In our study, the average tumor diameter of classical and pure ICC was 2.54 cm, which was slightly different from the previous study. A mass that is not easy to detect can also be found through mammographic findings [5, 12]. At the same time, the tumor can also be found by sonographic findings [12, 13]. Microcalcification was noted in 32% of the cases in imaging findings [8]. However, microcalcification can also occur in other breast lesions, therefore, it has been reported that it may not be used as diagnostic features. Previous studies have shown that the rate of axillary lymph node metastasis of ICC is lower than that of invasive ductal carcinoma (IDS) [2, 4, 6]. These studies have shown the rate of axillary lymph node metastasis was about 15.9%-25.5% [4, 6]. However, there is an individual report that shows a higher rate (55.6%) of lymph node metastases [10]. In addition, the number of axillary lymph node metastasis was not greater than three [2,
Compared to pure ICC, lymph node metastases were positive for ER and PR, and HER-2 only in a few cases [3, 4, 10]. In this study, all HER-2 expression was negative or expressed expression of ER and PR was positive, whereas immunohistochemical studies have shown the positive, and Her2 was negative) [4, 7, 9]. Early the ICCs were Luminal A (where ER and PR were is more common and easier to perform. Most of cer's immunohistochemical alternative typing in the routine work of pathology, breast can- is proposed. However, molecular classification of breast cancer based biological characteristics of breast cancer, the In order to gain a better understanding of the tion of breast cancer depends on routine paraffin section -scope. Therefore, the diagnosis and classifica- tion of a cribriform structure under the micro -scope. The key to the diagnosis of ICC is the observa- tions around the nest of the cancer are evident, even with varying degrees of collagenization. The key to the diagnosis of ICC is the observation of a cribriform structure under the microscope. Therefore, the diagnosis and classification of ICC depends on routine paraffin section and immunohistochemistry.

In order to gain a better understanding of the biological characteristics of breast cancer, the molecular classification of breast cancer based on gene array profiling was proposed. However, in the routine work of pathology, breast cancer's immunohistochemical alternative typing is more common and easier to perform. Most of the ICCs were Luminal A (where ER and PR were positive, and Her2 was negative) [4, 7, 9]. Early immunohistochemical studies have shown the expression of ER and PR was positive, whereas HER-2 expression was negative or expressed only in a few cases [3, 4, 10]. In this study, all cases were positive for ER and PR, and HER-2 was negative in all cases except in one case which was positive (2+), which is in agreement with the reported literatures. According to Zhang W et al. study [4], about 72.5% (37/51) of the ICC cases showed the tumor's proliferation index (ki67) being ≤ 14%. Meanwhile, the cases of pure ICC exhibited a lower proliferation index than the cases of mixed ICC. In our case, there were 58% (7/12) of the cases with a ki67 of ≤ 14%, and two of the three mixed ICC cases had a Ki67 of > 14%, which is basically consistent with the previous study. A lacking expression of myoepithelial markers such as SMA and p63 can help distinguish between the invasive components from the in-situ lesions [4]. In our case, the absence of these myoepithelial markers (calponin, p63, CK5/6 and CD10) around the cancer nest suggests that the tumor is invasive. On the contrary, in the internal control group, some of the myoepithelial markers' expression can be seen around the normal breast's ducts (Figure 2G, 2I). E-cadherin and P120 were positive in the tumor cells, suggesting the source came from the tubules, which is consistent with Page et al. who speculated that ICC was derived from the tubules or variants of the tubules [1]. Meanwhile, the new classification of breast cancer by the World Health Organization (WHO) even incorporates ICC and TC into a category [5]. In addition, in order to exclude the neuroendocrine differentiation, the other 3 cases were tested for neuroendocrine markers, such as CD56, CgA and Syn, and the results were all negative.

It is important to recognize this spectrum of histomorphology and differential diagnosis, as benign breast diseases or other invasive breast cancers may also show a cribriform pattern, such as collagenous spherulosis, cribriform ductal carcinoma in situ (DCIS), adenoid Cystic carcinoma, among others. Collagenous spherulosis is a benign breast hyperplasia disease, with accidental microscopic findings [15] and exhibiting rare occurrences of tumor formation, whereas in ICC, the tumor can be seen with the naked eye. The characteristics of the intracavity of collagenous spherulosis are transparent, containing cell-free eosinophilic collagen globules or fine amorphous substances. These intracavitary deposits consist of basal membrane components (type IV collagen and laminin), with the matrix and the epithelial cell nucleus having no atypia. The eosinophilic collagen globules are surrounded by muscle epi-
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In contrast, ICC has an Island-like infiltration pattern, with a lack of myoepithelium around the cancer nest and the nuclei have a light to moderate abnormality. Another pathological mimic of ICC is ductal carcinoma in situ (DCIS) which presents as non-invasive, has a smooth circular outline with myoepithelial cells surrounding the tubules and a lack of fiber response around the stroma. On the other hand, ICC has an irregular Island-shaped infiltration pattern or an angular infiltration pattern together with a lack of myoepithelium around the cancer nest. In addition, adenoid cystic carcinoma of the breast (ACCB) is also one of the differential diagnoses. ACCB is composed of a dual-cell population of inner luminal cells and outer myoepithelial-basal cells. The true gland lumina are lined up with ductal epithelial cells (EMA+, keratin+, CD117+) [16]. PAS staining was positive in the lumen. The pseudolumina due to its interstitial formation of invagination (interstitial cavity), Alcian blue staining, surrounded by basal myoepithelial cells (Vimentin+, P63+, Calponin+). In contrast to ACCB, although ICC has a similar morphology, it has only one cell type, with the disappearance of basal myoepithelial cells. In addition, ER and PR are usually negative with ACCB compared to ICC. Furthermore, ACCB generally has no DCIS component, while ICC is often composed of DCIS. As cribriform pattern can also be seen in neuroendocrine tumors, so the differential diagnosis of breast neuroendocrine cancer is also necessary. Eosinophilic granules can be found in the cytoplasm of neuroendocrine tumors, and the positive neuroendocrine markers CD56, CgA and Syn makes it easier to differentiate neuroendocrine tumors with ICC, since ICC responds negatively to these markers. One also needs to consider the possibility of a cribriform-patterned metastasis from other sites such as, salivary gland, colon, prostate, uterus or ovaries. In general, these metastases present with a high-grade cell morphology, and immunohistochemistry (such as CK20, CDX-2, PSA and PAX-8) is helpful for the differential diagnosis of the metastasis and in determining the site of origin [17]. Meanwhile, we should also pay attention to ICC when it appears in other forms, as some authors have reported a case of ICC that mimics the growth of abscesses clinically [18].

A number of studies have shown that the prognosis of ICC is better than that of invasive ductal carcinoma (IDC) [1, 2, 4, 6, 9]. The 10-year survival rate of classical ICC is 90% to 100%, and the 10-year survival rate of mixed ICC is worse than that of classical ICC, but it is still better than the prognosis of IDC [1, 9]. Venable et al. research shows that the 5-year survival rate of classical ICC is 100%, the 5-year survival rate of mixed ICC is 88%, and that of IDC is 78.3% [2]. Thus, from a histological or clinical point of view, ICC should be distinguished from IDC as a special type of breast cancer [2]. Based on the SEER database, Liu XY et al. [6] collected 618 cases of ICC and 232719 cases of IDC for comparison to analyze the clinicopathological characteristics of ICC, thus showing this special histologic type exhibits a lower grade, smaller tumor size, lesser lymph nodes involvement, earlier stage, higher positivity rate of hormone expression, and a lower HER2 amplification rate than IDC. Interestingly, after the multivariate Cox regression analysis adjustment for potential confounders, they found that the histological type of ICC is not an independent prognostic factor, which shows a little bias in the recognition of ICC as a special type of breast cancer with a good prognosis. It is reported that the good prognosis of ICC may be related to several factors such as the small size of the tumors, along with fewer lymph nodes metastasis, and expressing a positive ER and PR value with no amplification of Her2, and a low proliferation index of Ki-67 [7, 10]. In our case, all follow-up patients were disease-free survival except for one patient whom we lost during the follow up. Although mixed ICC has a remarkable amount of invasive ductal carcinoma or mucinous carcinoma, it still showed a good prognosis, and the overall prognosis cannot be distinguished from classical ICC or pure ICC. Therefore, an increase in the number of cases and the prolongation of the follow-up time may help to differentiate the prognosis of ICC from other types of breast cancer and perform a prognostic analysis of ICC subtypes. Inevitably, our study has a few limitations. Due to the small number of our cases and that all patients were tumor-free survival; we cannot carry out the analysis of the prognostic factors.

In conclusion, the present study described the clinicopathological and immunophenotypic characteristics of 12 cases of ICC of the breast. ICC is a rare histological subtype of breast cancer, with its unique characteristics, leading to a
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good prognosis. Mastering the diagnosis and differential diagnosis of ICC, in order to distinguish between the benign and malignant breast lesions, will be of great significance for the prognosis and treatment of the patients. In the future, larger samples and more follow-up times will be required for further study.

Disclosure of conflict of interest

None.

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