Breast Microinvasive Carcinoma With Different Morphologies: Analysis of Clinicopathologic Features of 121 Cases

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ABSTRACT

PURPOSE: To investigate the clinicopathological features of patients with breast microinvasive carcinoma (MI).

METHODS: The clinical data of 121 cases with breast MI were retrospectively collected. The whole tumor in each case was stained with hematoxylin and eosin (H&E) for pathological evaluation. The relationships among size of tumor, histological grade, tumor-infiltrating lymphocytes (TILs), the number of MIs, type of MI, and lymph node metastasis were analyzed.

RESULTS: It was revealed that 86% of the cases had high-grade ductal carcinoma in situ (DCIS) and 63.6% had multiple MIs. The larger size of the tumors, the higher the grade of DCIS, the more the number of MIs; 3.3% of cases had rich TILs (lymphocyte/stroma > 30%) in the DCIS, and 26.5% had rich TILs in MIs. The type A of MIs is characterized by single cells and small clusters of solid cells. Tumor cells in type B of MIs can form glandular ducts. Formal grading of microinvasive is challenging/impossible due to its limited size precluding a representative mitotic count. But nuclear grade and tubule (differentiation) grades can be reported. In addition, 72.7% of cases had type A of MIs and 27.3% of cases had type B of MIs. Type B was found to be highly accompanied by moderate-grade DCIS. Only 6.6% of patients with MI had lymph node metastasis, which was mainly related to MIs with less TILs.

CONCLUSION: Breast MI is easy to occur in high-grade DCIS, and multiple infiltration foci may be observed in case with tumor size of higher than 3.5 cm. Microinvasive carcinoma with poor TILs maybe a risk factor for lymph node metastasis in patient with DCIS-Mi.

KEYWORDS: Lymphocytes, tumor-infiltrating, breast carcinoma in situ, pathology, diagnosis

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Introduction

According to the guideline published by the American Joint Committee on Cancer (AJCC), breast ductal carcinoma in situ (DCIS)-Mi is defined as a breast DCIS with an infiltrative lesion less than 1 mm.[1] In routine clinical and pathological examinations, we found that there are 2 types of microinvasive carcinoma (MI). Microscopically, the type A MI of the breast is characterized by a single tumor cell penetrating the myoepithelium and basement membrane in the form of "budding," which indicates that type A MI may be closely related to carcinoma in situ (CIS). The infiltrated tumor cells can be isolated and scattered in the stroma. And the multiple infiltrated tumor cells can also gather together to form cell clusters, but they cannot form the glandular tube with lumen. The nuclear grade of infiltrated tumor cells can be low or high grade. The nuclear grade of CIS is often high grade. This type of MI often has more tumor-infiltrating lymphocytes (TILs).

Microscopically, the type B MI of the breast is characterized by several small glandular ducts surrounding the CIS. These small glandular ducts lack myoepithelium and have medium-sized nuclei and few mitotic figures. Therefore, these glandular ducts are invasive carcinoma. These glandular ducts often have a certain space distance from the ducts of CIS, which indicates that they may not be closely related to CIS. This type of MI has only a small number of TILs.

De Mascarel et al divided the MI into 2 types: type I carcinoma cells infiltrated into the stroma outside the basement membrane as a single focus, with a cell number of 1 to 15; type II carcinoma cells infiltrated into the stroma outside the basement membrane as a cluster.[2] This classification is only based on the number of cells and does not include type B MI proposed by the author of this text. No clear pathological classification has been proposed yet. In addition, the clinicopathological significance of these 2 types has still remained elusive.

In the present study, 121 cases of breast DCIS-Mi were collected. The pathological features of those cases were discussed, including the tumor size, the grade of breast DCIS, comedo-like necrosis, the number of MIs, type of MIs, the number of TILs around breast DCIS(TILs-DCIS), the number of TILs in MIs(TILs-MIs), and axillary lymph nodes. To improve the recognition of such diseases by pathologists, summarizing the clinicopathological characteristics and further enhancement of understanding this type of tumor are highly essential.

Study Subjects and Methods

Study subjects

In the present study, 121 cases with breast DCIS-Mi who were diagnosed by pathology in Fujian Medical University Union Hospital during January 2016 to December 2018 were recruited; 6071 cases of invasive carcinoma and 53 cases (0.87%) of CIS have been diagnosed at the same time. The incidence of microinvasive cancer is 1.9%. All 121 cases are based on excision specimens. All cases were reexamined by 2 experienced senior pathologists. The diagnostic criteria were based on a previously conducted research, and the improved Scarff-Bloom-Richardson grading system was used to classify CIS.[3] The characteristic of low-grade CIS is that the nucleus is slightly larger than that of normal breast ductal epithelium, and the nucleus is the same size. The tumor cells form cribriform structure in the breast duct without tumor necrosis. The characteristic of high-grade CIS is that the nucleus of tumor is three times larger than that of normal ductal epithelium. The tumor cells grow solid in the duct, and the central area often has comedo-like necrosis. The morphologic characteristics of intermediate-grade CIS are between the 2. The nucleus of tumor is 2 to 3 times larger than that of normal ductal epithelium. In cases of availability of discrepancy between the 2 pathologists' statement, the third pathologist's decision was confirmed. All clinical data were obtained from the electronic medical records. All patients received mastectomy. Patients with lymph node metastasis also received chemotherapy.

Processing of tissue specimens

All specimens were fixed with 10% neutral formalin. The suspected tumors were observed by naked eyes, and the whole tumors were made into $2 \times 1.5 \times 0.2$ size paraffin-embedded tissue blocks. Paraffin-embedded tissue blocks were cut into sections with thickness of 4 μm and then stained with hematoxylin and eosin (H&E). The suspicious MIs examined by H&E were further clarified by immunohistochemical staining of myoepithelial cells.

Myoepithelial markers anti-P63(MX013), SMMHC (SMMS-1), ER(SP1), and PR(SP2) were purchased from Maixin Biotechnology Development Co., Ltd. (Fuzhou, China). HER2(4B5) were purchased from Roche Pharma ceutical Ltd. (Switzerland). Immunohistochemistry was performed as previously described.[4] Antigen retrieval was performed by pressure cooking in citrate buffer (0.01 mol/L; pH 6.0) after the tissue sections were dewaxed and then soaked in 3% H_2O_2 for 10 min. The primary antibody was then added to the treated tissue sections and incubated at room temperature for 2 hours, followed by Horseradish Peroxidase Reaction Detection kit with DAB chromogen (Elivision™ plus Polymer HRP Mouse/Rabbit IHC kit-9902; Maixin Company, Fuzhou, China) as per the manufacturer's instructions. The sections were washed with phosphate-buffered saline (PBS; 0.01 M, pH 7.2) throughout the above steps. We used PBS as negative control. Hematoxylin was applied as a counterstain. The evaluation of ER, PR, HER2, and molecular subtypes was according to the literature.[5,6]

The counting methods and morphological classification criteria for MIs

The number of MIs in all sections was counted. The MIs in different sections were defined as non-unique MIs, and the MIs in different CIS in the same section were defined as one MI. Morphological classification of MIs: type A: cancer cells infiltrated outside basement membrane, in clusters, with solid tumor cell clusters, without ductal structure; type B: infiltration of cancer cells formed ductal structure.

Evaluation of TILs

As described previously,[7] the numbers of lymphocytes in the DCIS and in the MIs were counted separately. The proportion of lymphocytes in the stroma was assessed as follows: 0: 0%-9%, 1: 10%-29%, 2: 30%-49%, 3: 50%-100%.

Statistical analysis

In the present research, SPSS 17.0 software (IBM, Armonk, NY, USA) was used to perform statistical analyses. Chi-square test was used for comparing categorical variables. Logistic regression analysis was used to obtain odds ratios (ORs) in the presence of more than one explanatory variable. $P < .05$ was considered statistically significant.

Results

Clinical features

All the 121 cases with breast DCIS-Mi were female with a median age of 47 years, range = 29 to 84 years. In addition, 81 cases (66.9%) were younger than 50 years, and 40 cases (33.1%) were older than 50 years; 68 cases (56.2%) were left breast DCIS-Mi, and 53 cases (43.8%) were right breast DCIS-Mi. The average size of tumors was 3.41 cm, range = 1.8 to 10 cm. It also was uncovered that in 82 cases (67.8%), the size of tumors was less than 3.5 cm and that was higher than 3.5 cm in 39 cases (32.2%). The average number of paraffin-embedded tissue blocks produced in each case was 32, range = 6 to 110.

Pathological examination

Most cases have comedo-like necrosis in the duct. In some cases, braided nodules or cysts with different sizes were noted in the surrounding mammary glands.

Microscopic examination revealed that DCIS was present in all the cases. Immunohistochemical staining showed the presence of surrounding myoepithelial markers (anti-P63, SMMHC, calponin positive). In addition, 17 cases (14%) had moderate-grade

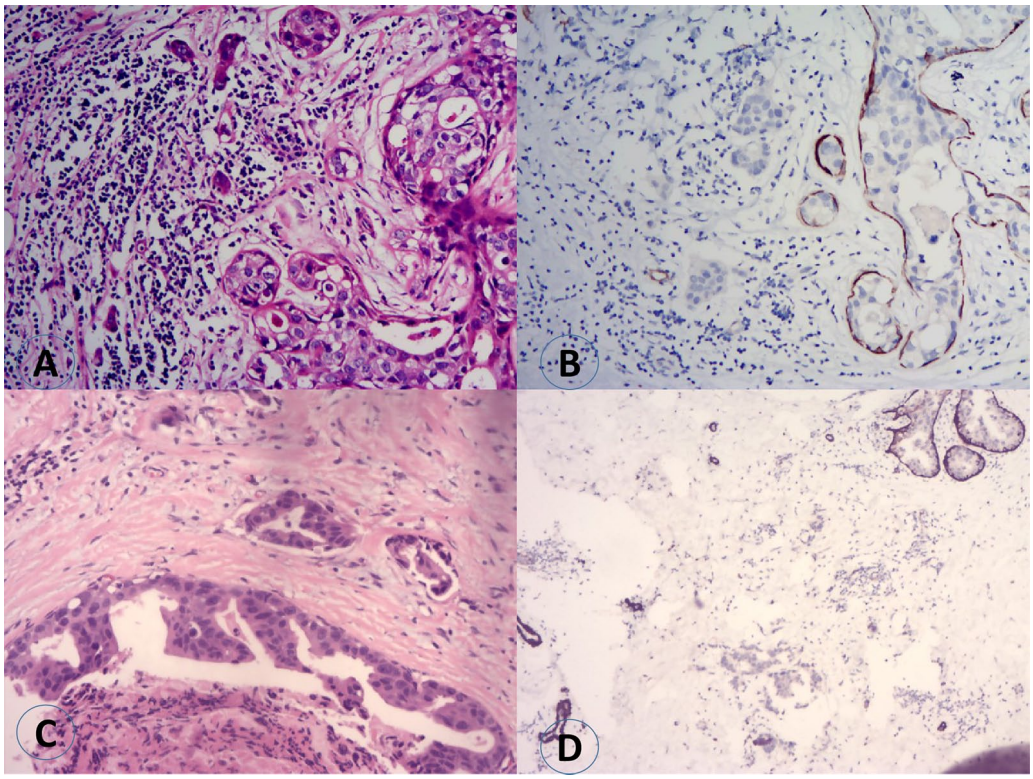

Figure 1. Type A microinvasive carcinoma of the breast (A and B): (A) scattered single cells and clusters of tumor cells around high-grade ductal carcinoma in situ, with infiltrating lymphocytes (H&E staining). (B) Immunohistochemical staining of SMMHC, a myoepithelial marker, showed that there was no myoepithelial cell surrounding the scattered tumor cells in (A). Type B microinvasive carcinoma of the breast (C and D): (C) several small glandular ducts around the middle-grade ductal carcinoma in situ and no lymphocyte around small glandular ducts (H&E staining). (D) Immunohistochemical staining of SMMHC, a myoepithelial marker, showed that there was no myoepithelial cell surrounding the small glandular ducts in (C). H&E indicates hematoxylin and eosin; SMMHC, smooth muscle myosin heavy chain.

DCIS and 104 cases (86%) had high-grade DCIS; 66 cases (54.5%) had comedo-like necrosis. 45 cases (36.4%) had only one MI, and 76 cases (63.6%) had 2 MIs or more than 2 MIs. It was also found that 88 cases (72.7%) had type A of MIs and 33 cases (27.3%) had type B of MIs (Figure 1A to D).

The TILs-DCIS score was 0 in 69 cases, 1 in 48 cases, 2 in 3 cases, and 3 in one case. The TILs-MIs score was 0 in 38 cases, 1 in 51 cases, 2 in 18 cases, and 3 in 18 cases. Only 4 cases (3.3%) had high TILs-DCIS (lymphocyte/stroma >30%), while 32 cases (26.5%) had high TILs-MIs.

Other lesions of breast cancer were as follows: 3 cases showed microinvasive lesions invading nerve fibers, and 1 case showed intravascular tumor emboli. One case had solid papillary CIS, and 1 case had apocrine CIS. Paget disease was found in 7 cases and lobular CIS in 3 cases; 6 cases had fibrocystic, 6 cases were sclerosing adenosis, 8 cases had fibroadenoma, and 11 cases had intraductal papilloma.

Sentinel lymph nodes were examined in all the cases. Tumor cells were found in 6.6% of cases, including isolated tumor cells (ITC) in 4 cases, micrometastasis in 3 cases, and macrometastasis in 1 case.

Because of the small size of the microinvasive lesions, this study only evaluated the hormone status of CIS. The positive rate of ER was 45.5%. The positive rate of PR was 42.1%. The positive rate of HER2(2+/3+) was 43.1%. The molecular types of breast cancer were as follows: 34 cases (28.1%) were type A, 21 cases (17.4%) were type B, 28 cases (23.1%) were HER2 overexpression, and 38 cases (31.4%) were basal-like.

The relationship between the number of MIs and clinicopathological features

According to the TILs in DCIS, the patients were divided into 2 groups: Negative group (score of TILs = 0) and Positive group (score of TILs > 0). According to the number of MIs, all the cases were categorized into single MI group and multiple MIs group. The patients were then divided into 2 groups according to the number of TILs in the MI: a low group with 0 to 1 points and a high group with 2 to 3 points.

Chi-square test showed that the number of MIs was associated with the size of tumors, the grade of DCIS, and the number of TILs in MIs. Compared with single MI, multifocal MIs had a wider range of primary tumors, more advanced primary tumors, and further TIL cells. The difference was statistically significant (all $P < .05$, Table 1). The number of MIs was not correlated with patients' age, location of tumors, comedo-like necrosis, and the number of TILs, ER, PR, HER2, and molecular types ($P > .05$, Table 1).

Table 1. The relationship between MIs and clinicopathological parameters in 121 patients with breast DCIS-Mi.

CLINICOPATHOLOGICAL PARAMETERS	(N, %)	NUMBER OF MIS (N, %)		*P*	TYPE OF MIS (N, %)		*P*
		SINGLE	MULTIPLE		A	B	
Age (years)				.250			.407
⩽50	81 (66.9)	33 (73.3)	48 (63.2)		57 (64.7)	24 (72.7)	
>50	40 (33.1)	12 (26.6)	28 (36.8)		31 (35.3)	9 (27.3)	
Site				.178			.067
Left	68 (56.2)	25 (55.6)	43 (56.6)		45 (51.1)	23 (69.7)	
Right	53 (43.8)	20 (44.4)	33 (43.4)		43 (48.9)	10 (30.3)	
Tumor size (cm)				**.027**			.874
⩽3.5	82 (67.8)	36 (80.0)	46 (60.5)	**.046**[a]	60 (68.2)	22 (66.7)	
>3.5	39 (32.2)	9 (20.0)	30 (39.5)		28 (31.8)	11 (33.3)	
Grade of DCIS				.002			.010
Moderate	17 (14.0)	12 (26.7)	5 (6.6)	**.017**[a]	8 (9.1)	9 (27.3)	**.011**[a]
High	104 (86.0)	33 (73.3)	71 (93.4)		80 (90.9)	24 (72.7)	
Comedo-like necrosis				.336			.412
Negative	55 (45.5)	23 (51.1)	32 (42.1)		38 (43.2)	17 (51.5)	
Positive	66 (54.5)	22 (48.9)	44 (57.9)		50 (56.8)	16 (48.5)	
Number of MIs							**.046**
Single	45 (36.4)				28 (31.8)	17 (51.5)	
Multiple	76 (63.6)				60 (68.2)	16 (48.5)	
TILs of DCIS				.205			.114
Negative	69 (57.0)	29 (64.4)	40 (52.6)		51 (73.9)	18 (54.5)	
Positive	52 (43.0)	16 (35.6)	36 (47.4)		37 (71.2)	15 (45.5)	
TILs of MIs				**.037**			**.029**
Low	89 (73.6)	38 (84.4)	51 (67.1)		60 (68.2)	29 (87.9)	
High	32 (26.4)	7 (15.6)	25 (32.9)		28 (31.8)	4 (12.1)	
ER				.852			.688
Negative	66 (54.5)	24 (53.3)	42 (55.3)		49 (55.7)	17 (51.5)	
Positive	55 (45.5)	21 (46.7)	34 (44.7)		39 (44.3)	16 (48.5)	
PR				.849			.565
Negative	70 (57.9)	27 (60.0)	43 (56.6)		51 (58.0)	19 (57.6)	
Positive	51 (42.1)	18 (40.0)	33 (43.4)		37 (42.0)	14 (42.4)	
HER2				.126			.880
0/1+	71 (58.7)	22 (48.9)	49 (64.5)		52 (59.1)	19 (57.6)	
2+/3+	50 (41.3)	23 (51.1)	27 (35.5)		36 (40.9)	14 (42.4)	
Type				.529			.980
Lumina A	34 (28.1)	11 (24.4)	23 (30.3)		24 (27.3)	10 (30.3)	
Lumina B	21 (17.4)	10 (22.2)	11 (14.5)		15 (17.0)	6 (18.2)	
HER2+	28 (23.1)	12 (26.7)	16 (21.1)		21 (23.9)	7 (21.2)	
Basal-like	38 (31.4)	12 (26.7)	26 (34.2)		28 (31.8)	10 (30.3)	

Abbreviations: DCIS, ductal carcinoma in situ; MI, microinvasive carcinoma; ER, estrogen receptors; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.
[a]Multivariate logist regression analysis of *P* value.
Bold values denotes $P < 0.05$.

Table 2. The relationship between MIs and lymph node metastasis in 121 cases with breast DCIS-Mi.

CLINICOPATHOLOGICAL PARAMETERS	THE STATUS OF LYMPH NODE METASTASIS			*P*
	NO TUMOR CELL	ITC	MICROMETASTASIS/ MACROMETASTASIS	
Number of MIs				.250
Single	44	1	0	
Multiple	69	3	4	
Type of MIs				.582
A	83	2	3	
B	30	2	1	
TILs of MIs				.263
Low	81	4	4	
High	32	0	0	

Abbreviations: DCIS, ductal carcinoma in situ; ITC, isolated tumor cells; MI, microinvasive carcinoma.

Multivariate logistic regression analysis showed that the size of tumor and the grade of DCIS were independent risk factors, influencing the number of MIs (*P* values were .046 and .017, respectively). The larger size of the tumors, the higher the grade of DCIS, the more the number of MIs (Table 1).

The relationship between type of MIs and clinicopathological features

Chi-square test revealed that the type of MIs was correlated with the grade of DCIS, the number of MIs, and the number of TILs in MIs. In addition, 9.1% of cases in type A were accompanied by moderate-grade DCIS, while 27.3% in type B were accompanied by moderate-grade DCIS. In addition, 31.8% of the cases with type A had single invasive focus, while 51.5% of the cases with type B had single invasive focus. In type A, 31.8% of TILs are lymphocyte rich, while in type B, 12.1% of TILs are lymphocyte rich ($P < .05$). The type of MIs was not correlated with the patients' age; the location of tumor; the size of tumor; comedo-like necrosis; the number of TILs in DCIS, ER, PR, and HER2; and molecular types ($P > .05$).

Multivariate logistic regression analysis uncovered that the grade of DCIS was an independent risk factor, affecting the type of MIs ($P = .011$). Type B was more likely accompanied by intermediate-grade DCIS (Table 1).

The relationship between MIs and lymph node metastasis

Tumor cells were found in only 7 cases with sentinel lymph nodes; of whom, cases had multiple MIs in breast tumor. Lymph node metastasis can occur in all types of MIs. The lymph node metastasis is more likely to occur in patients with less TILs. There was no significant difference in lymph node metastasis between different types of MIs (Table 2).

Discussion

With the introduction of population-based mammographic screening programs, there has been an increased detection of putative precursor lesions. While the detection of invasive ductal carcinoma (IDC) by mammographic screening programs has increased 1.6-fold, the detection of benign lesions has increased 2- to 4-fold, indicating that not all precursor lesions will ever progress to malignancy.[8,9] In addition, DCIS-Mi has been defined as the maximum diameter of infiltrative lesions (<1 mm); if there are multiple infiltrative lesions, the maximum diameter of each one should be less than 1 mm.[2] According to the literature, the incidence of DCIS and DCIS-Mi is 1.3% and 12.1%.[10] But in this study, we found that the proportion of pure CIS is not so high. The proportion of microinvasive cancer is higher. This may be due to the fact that all the visible tumors in this study were made into pathological sections and observed under microscope.

A number of studies reported that cases of breast DCIS-Mi had larger tumor size than CIS.[11,12] Chen et al[13] found that interstitial infiltration was prone to occur in cases with breast DCIS, in which diameter of tumor was larger than 3.15 cm. In the present study, the diameter of tumor in 75% of cases was larger than 2.1 cm. It is recommended that not only high-grade DCIS, but intermediate-grade DCIS also can also be associated with microinvasion, and that this can be associated with nodal metastasis in rare cases. For patients whose diameter of tumor is higher than 3.5 cm, multiple MIs are more likely to occur. Therefore, adequate sampling should be made to prevent missing the largest infiltrating foci, as well as reducing the staging of tumors. Breast tumors with high-grade DCIS are more susceptible to microinvasion.[14] In the present research, we found that high-grade DCIS is not only prone to MIs but also up to 93.4% of cases with multiple MIs had high-grade DCIS. In a study conducted by Kim et al,[12] 30% of DCIS-Mi patients (41/136) had multiple infiltrative lesions, and there was no significant

difference in the patients' clinicopathological characteristics between single infiltrative lesion and multiple infiltrative lesions. However, in the current research, 63.6% of patients with DCIS-Mi had multiple infiltrative lesions, and the former tumor size with multiple infiltrative lesions was larger than that of the single-invasive lesion. Multifocal MI may be a risk factor for local recurrence of tumors in such patients as well.[15]

De Mascarel et al[2] classified DCIS-Mi into type I and type II according to the size of the infiltrating focus. In the present study, we found that there are 2 conditions of type II of infiltration foci: one is that the tumor cells are bulky and budding and the other is that the tumor has obvious ductal structure, similar to well-differentiated invasive ductal cancer structure, which we defined it as Type B. This type of MI is different from Type A. The majority of cases with Type B of MIs have single lesions, and there are a limited number of TILs in MIs.

TILs in invasive breast cancer have a great impact on the prognosis and treatment response of patients and can be used as a predictor of response to immunotherapy.[16] However, the role of TILs in both in situ and MIs has still remained elusive. Beguinot et al analyzed 96 cases of pure DCIS and 35 cases of microinvasive breast cancer. It was found that microinvasive breast cancer had more TILs. The biological behavior of TILs-rich DCIS (TILs > 30%) was similar to that of DCIS-Mi. Periductal infiltration of lymphocytes was a risk factor for tumor infiltration.[7] In the present study, the number of TILs in lesions was greater than that in DCIS. The number of infiltrative lesions is not related to the number of TILs in DCIS, while is closely associated with the number of TILs in infiltrative lesions.

In this study, the positive rates of ER and PR were lower than those reported in the literature, while the positive rates of HER2 were higher than those reported in the literature, which may be related to race, or the proportion of high-grade DCIS in this study was higher.[17] In this study, there was no difference in hormone status between the 2 forms of MI.

The incidence of lymph node metastasis in microinvasive breast cancer was 5.5%-12%.[10,18,19] The proportion of lymph node metastasis was 6.6% (8/121) in the present study, which was similar to that reported in the literature. At the same time, the size of CIS reported in the literature is similar to the tumor size range in this study. The cases with lymph node metastasis were those with multiple MIs. The number of TILs in MIs is relatively small, which may indicate that tumor cells evade the monitoring of the immune system. Such cases are more likely to have lymph node metastasis.

Because the MI of the breast is very small, it is theoretically impossible to rule out that some microscopic invasive lesions in this study were not detected. At the same time, the study is only a single-center study, so it depends on more cases for further research.

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Author Contributions

FC designed the research study, performed pathological evaluation, and wrote the paper. ZQ performed research and analyzed the data. YY performed the pathological evaluation.

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