

Research Article

Is The Diagnosis of Carcinoma with Neuroendocrine Features of The Breast Possible in Fine-Needle Aspiration Specimens?

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Keywords

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Abstract

Several previous studies focused on the ability of fine-needle aspiration cytology (FNAC) to diagnose carcinomas with neuroendocrine features (CNF). A pre-operative cytological diagnosis of malignancy was made in all seven CNF cases in the present study. However, we failed to accurately subtype most of these breast carcinoma cases. Five cases were reported as invasive carcinoma of no special type, one as intracystic carcinoma, and only one as neuroendocrine DCIS suggested. The expectation that FNAC methods can reliably make similar diagnoses as a histopathological diagnosis on morphological grounds alone may be overly optimistic at present. The accuracy of a FNAC diagnosis in the management of CNF depends on multiple approaches including the immunohistochemical staining of surgical specimens.

ABBREVIATIONS

FNAC: fine-needle aspiration cytology; **CNF:** carcinoma with neuroendocrine features, **DCIS:** ductal carcinoma *in situ*

INTRODUCTION

Fine-needle aspiration cytology (FNAC) is a well-established method for the diagnosis of breast lesions [1, 2]. Several previous studies focused on the ability of FNAC to diagnose carcinomas with neuroendocrine features (CNF) [3-12]. As FNAC has been a sensitive technique for diagnosing breast carcinoma, all of these studies have reported some degree of difficulty. Despite previous studies reporting the inability of FNAC to diagnose CNF, efforts to reevaluate this issue have been made sporadically. However, most of these studies were single case reports or only represented a small number of cases. The aim of this study was to examine the possibility of using FNAC specimens to distinguish CNF from other types of tumors by comparing cytomorphological features. This study evaluated the clinical features and morphology of breast CNF and examined the diagnostic utility of FNAC.

MATERIALS AND METHODS

We retrospectively reviewed the histology records of CNF lumpectomy cases at the Department of Pathology, International University of Health and Welfare Hospital, Department of Pathology, International University of Health and Welfare Mita Hospital, and NissanTamagawa Hospital between 2001 and 2014. All CNF cases were reclassified according to the World Health Organization 2012 classification scheme for breast tumors. Based on these criteria, 7 patients were judged to have CNF by histological specimens. The diagnostic results of FNAC and clinical records were searched for in computer files or medical charts. Immunohistochemistry data were available for all 7 cases of histological specimens. All antibodies were prediluted and provided by NICHIREI BIOSCIENCES INC. Representative blocks were then selected for immunostaining. Synaptophysin (SYN), ChromograninA (CgA), CD56, and Neuron-specific enolase (NSE) were evaluated by an immunohistochemical analysis using a specific antibody. We examined the expression of proteins using a fully automated system (NICHIREI BIOSCIENCES INC

Histostainer 36A). Briefly, five- μ m-thick unstained sections were placed onto an electrostatically charged glass slide and baked to allow for tissue adherence. The glass slides were pretreated with the recommended pretreatment solution provided by the fully automated system for antigen retrieval. Negative controls were obtained by omitting the positive controls. The immunohistochemically stained slides of each tumor were compared with positive and negative controls.

RESULTS AND DISCUSSION

The clinical characteristics of CNF are shown in Table 3. All seven patients with CNF were Japanese females with a mean age of 70.4 years (range 53-81 years). The clinical presentation was a palpable mass in five cases, while one case was detected by breast cancer screening and another by a bloody nipple discharge. The median follow-up for patients with CNF of the breast was examined in all cases and was 46 months (range, 24 to 84 months). No patient had recurrence or died of the disease after the initial diagnosis. The cytological characteristics of CNF are shown in Tables 1 and 2. Among the FNAC specimens from seven patients with CNF, all cases were adequate for diagnosis. A pre-operative cytological diagnosis of malignancy was made in all cases. However, we failed to accurately subtype most cases of breast carcinoma. Of these cases, five were reported as invasive carcinoma of no special type, one as intracystic carcinoma, and one as neuroendocrine ductal carcinoma *in situ* (DCIS) suggested, as there were only a few carcinoma cells in our FNAC specimens and because of rareness. The background was clear in four cases, colloid in two cases, and hemorrhagic in one case. At a low power view, FNAC of the tumors revealed a markedly cellular specimen in all cases (Figure 1). Three-dimensional loosely cohesive clusters of neoplastic cells and single dissociated neoplastic cells were frequently observed around the clusters. Most tumor cells were relatively small, round, oval polygonal, or spindle with an abundant cytoplasm with granules and round-to-oval nuclei with a fine granular chromatin pattern and inconspicuous nucleoli (Figure 2). The nuclear grade was estimated to be low in six cases and intermediate in one case. Rare mitoses, but no apoptosis or necrosis were identified. Neoplastic cells focally consisted of plasmacytoid features (Figure 3).

The histological and clinical characteristics of CNF are shown in Tables 1 and 3.

Pathological staging revealed one Tis, four T1, one T2, and one T3 category tumors. Solid nests of uniform tumor cells infiltrated a dense collagenous stroma (Figure 4, 5). The histological differentiation grades of the tumors were six of grade I and one of grade II. Five of the patients had no lymph node metastasis while two patients had metastasis to the lymph nodes. All tumors expressed SYN (Figure 6a). Three tumors expressed CD56 and CgA (Figure 6b,c). Six cases expressed NSE (Figure 6d). In one out of the seven cases, the tumor expressed all four neuroendocrine markers. Three cases expressed three markers while three cases expressed two markers.

FNAC is an established, highly accurate method for diagnosing breast lesions. However, previous studies insisted that the histological typing of breast carcinomas on FNAC was not possible, except on rare occasions [13]. However, the current

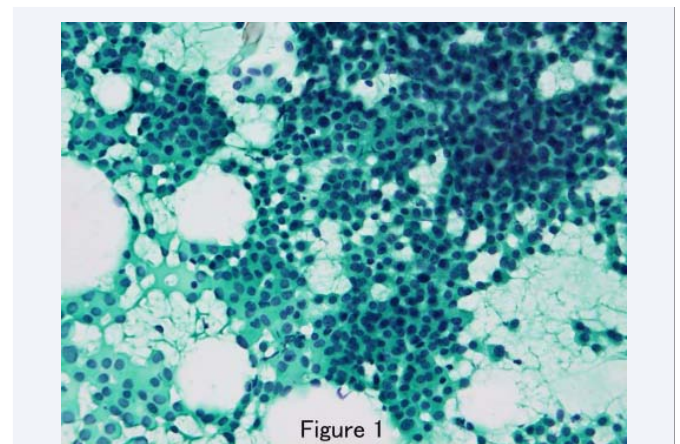


Figure 1 A highly cellular smear shows cells arranged in cohesive clusters and dispersed cells at a low-power magnification (Papanicolaou stain, x200).

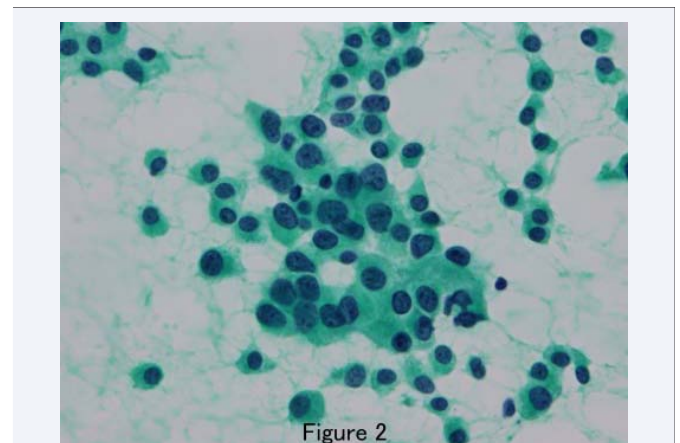


Figure 2 Cells were relatively large, round, or oval polygonal with an abundant cytoplasm with granules and eccentrically placed, pleomorphic nuclei with a fine granular chromatin pattern and inconspicuous nucleoli at a high-power magnification (Papanicolaou stain, x400).

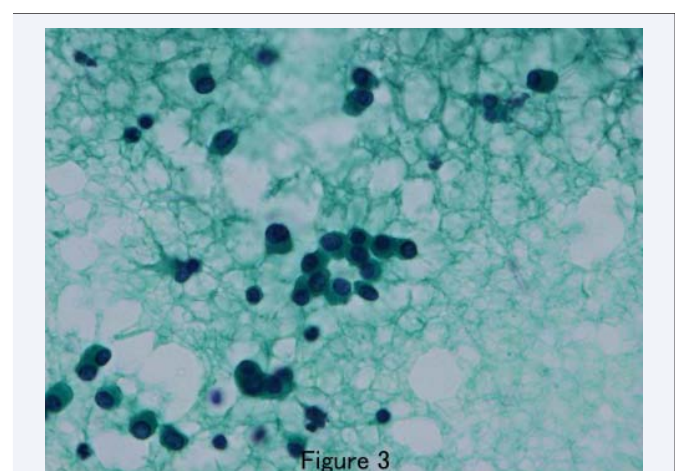


Figure 3 Isolated, fairly uniform tumor cells demonstrating a plasmacytoid appearance (Papanicolaou stain, x400).

Table 1: Cytological diagnoses Vs histological diagnoses of carcinomas with neuroendocrine features.

Case no.	Cytological diagnosis	Histological diagnosis
1	Neuroendocrine DCIS suggested	Neuroendocrine DCIS
2	Intracystic carcinoma	Neuroendocrine tumor, well differentiated
3	Invasive carcinoma of no special type	Neuroendocrine DCIS
4	Invasive carcinoma of no special type	Neuroendocrine tumor, well differentiated
5	Invasive carcinoma of no special type	Neuroendocrine tumor, well differentiated
6	Invasive carcinoma of no special type	Neuroendocrine tumor, well differentiated
7	Invasive carcinoma of no special type	Neuroendocrine tumor, well differentiated

DCIS: ductal carcinoma *in situ*

Table 2: Cytological features in seven cases of carcinomas with neuroendocrine features.

Case no.	Back ground	Cellularity	Cell clusters	Cell size	Cytoplasmic granules	Nuclear pleomorphism	Nuclear chromatin	Nucleoli
1	Hemorrhagic	High	Cohesive	Small	Present	Low	Fine	Inconspicuous
2	Colloid	High	Cohesive	Small	Present	Low	Fine	Inconspicuous
3	Colloid	High	Cohesive	Small	Present	Low	Fine	Inconspicuous
4	Clean	High	Cohesive	Small	Present	Low	Fine	Inconspicuous
5	Clean	High	Loose	Small	Present	Low	Fine	Inconspicuous
6	Clean	High	Loose	Small	Present	Low	Coarse	Obvious
7	Clean	High	Loose	Intermediate	Present	Intermediate	Coarse	Obvious

Table 3: Clinical and pathological data in seven cases of carcinoma with neuroendocrine features.

Case no.	Age	Side	Size	Histological grade	Lymph node status	Vessel invasion	SYN	CD56	CgA	NSE
1	63	Left	4.5	I	-	-	+	+	-	+
2	66	Right	6.5	I	-	-	+	-	-	+
3	74	Left	1.2	I	-	-	+	+	-	+
4	81	Right	1.5	I	-	-	+	+	+	+
5	76	Right	0.8	I	-	-	+	-	+	-
6	53	Left	1	I	+ (1/3)*	+	+	-	+	+
7	80	Left	2	II	+ (1/2)**	+	+	-	-	+

SYN: Synaptophysin CgA: Chromogranin A NSE: Neuron-specific enolase
 Size of the metastatic carcinoma *20mm in diameter **3mm in diameter

prevailing opinion in the field regarding the usefulness of FNAC may identify some tumor subtypes [1,2]. CNF is a complex entity of breast lesions that represents <1% of breast carcinomas [14]. In FNAC samples, CNFs are characterized by small uniform blue round epithelioid or spindle cells arranged in loosely cohesive clusters with a fine chromatin pattern and a lower degree of pleomorphism [15]. Low-grade CNFs typically comprise a uniform population of plasmacytoid or spindle cells, whereas a higher degree of pleomorphism, nuclear molding, nuclear streaking artifacts, and a necrotic background may suggest high-grade CNFs. However, due to its rarity, non-specific symptomatology, and architectural similarities to conventional variants of breast cancer, it has been proposed to be a diagnostic challenge in clinical practice [16]. In FNAC, the cytological appearance of the neoplastic cells of lobular carcinoma of the breast may be difficult to differentiate from those of mammary carcinoma with neuroendocrine features. Characteristically lobular carcinoma presents relatively dispersed discohesive tumor cells with a plasmacytoid morphology and occasional cytoplasmic vacuolization [17]. Furthermore, malignant lymphoma is another

possibility to consider in a differential diagnosis. A monotonous appearance and single cells appearing to resemble lymphoma, in which some tumor cells contained eccentric nuclei, suggest plasmacytoid features [18]. However, clusters of tumor cells do not exist in lobular carcinoma or lymphoma, but are typically present in FNAC material specimens of ductal carcinoma. Due to the above reasons, we succeeded in excluding the diagnosis of lymphoma and lobular carcinoma from our series.

The presence of a mucoid-like background is essential for the diagnosis of mucinous carcinoma, which has been reported in other subtypes of breast carcinoma [19]. Mucinous carcinomas of the breast appear to be associated with neuroendocrine differentiation. The pure forms of mucinous carcinomas were further subdivided into types A, B, and AB by Capella et al [20]. In that study, mucinous carcinoma type B with large cell clusters showed frequent neuroendocrine differentiation. Therefore, pathologists have to consider the possibility of CNF in the case of a mucoid-like background and apparent cytoplasmic granules. Furthermore, in our series of CNF, the presence of a mucoid-like

background was detected in two cases, however, histological confirmation was not mucinous carcinoma in either case. One limitation of FNAC is the difficulty in equating cytomorphologic features with the histological classification that is commonly used as the gold standard [21]. It is also difficult to distinguish between invasive lesions and *in situ* carcinoma [22]. In the present study, we succeeded in diagnosing CNF *in situ* in only one case. Kawasaki et al. analyzed 26 FNAC specimens obtained preoperatively from *in situ* CNF [23]. Of these cases, a preoperative cytological diagnosis of malignancy was only made in 11 cases (42%). Similar to our results, of the 11 cases diagnosed as malignant, seven were reported as ductal carcinoma and four as mucinous carcinoma. However, none of the cases were suggested to be CNF by FNAC. The majority of cases of CNF *in situ* have smaller, less atypical cells, and a fine chromatin nucleus with inconspicuous nucleoli. However, a sufficient overlap has been reported in the cytological findings between DCIS and CNF *in situ*, which makes any distinction between the two on FNAC indefinite.

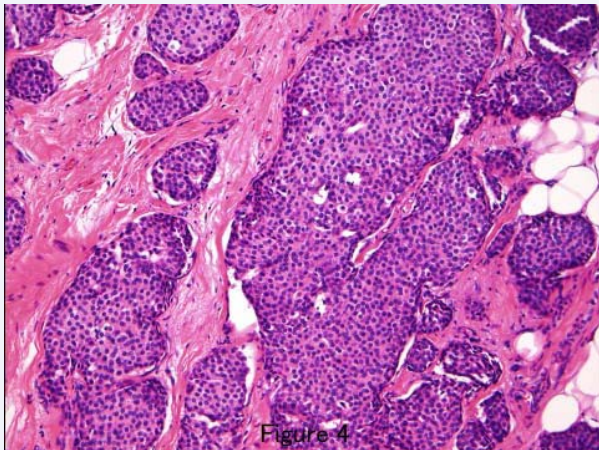


Figure 4 Solid nests infiltrating a dense collagenous stroma at a low-power magnification (HE x100).

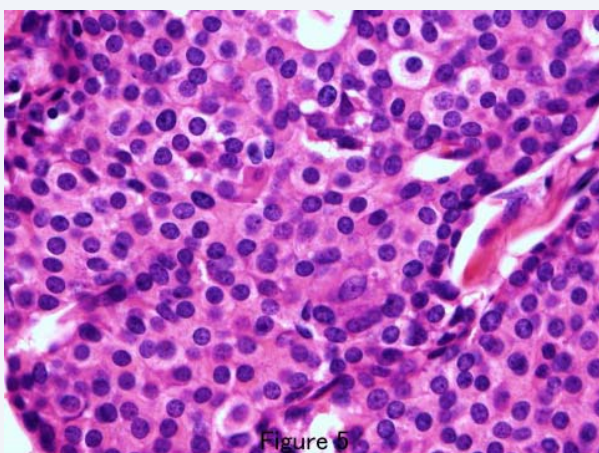


Figure 5 Cells were relatively small, round, oval, polygonal, or spindle-shaped with a cytoplasm with granules and round-to-oval nuclei with a fine granular chromatin pattern and inconspicuous nucleoli (HE x400).

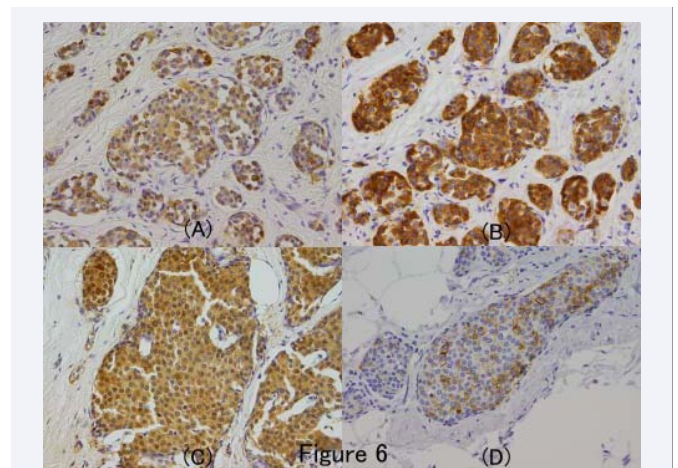


Figure 6 Immunohistochemical stains that were positive for (A) chromogranin A, (B) synaptophysin, (C) neuron-specific enolase, and (D) CD56 (x200).

Several previous studies reported the importance of neuroendocrine marker cut-off values [24-30]. Sapino et al. proposed that endocrine breast carcinomas expressed neuroendocrine markers in more than 50% of their cells. This parameter has since become a defining aspect of endocrine breast carcinomas in the most of the criteria used for a diagnosis of CNF [24]. However, in 2012, the World Health Organization (WHO) recommended the classification of CNF expressing neuroendocrine markers to a greater or a lesser degree and deleted the cut-off values [14]. They also insisted that since neuroendocrine markers were not routinely used on breast tumors, the true incidence of CNF is difficult to assess. This WHO recommendation indicates other types of breast carcinomas will partly share the same morphological structure of CNF. FNAC is not suitable for the accurate diagnosis of breast CNF due to its inability to estimate the entirety of the tumor lesion. Thus, cytological features in some cases may not be sufficient to impart a definite diagnosis, regardless of the pathologist's diagnostic ability.

CONCLUSION

The expectation that FNAC methods can reliably make similar diagnoses to a histopathological diagnosis on morphological grounds alone may be overly optimistic at present. The accuracy of FNAC diagnosis in the management of CNF depends on multiple approaches including the immunohistochemical staining of surgical specimens.

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