

Anna Sapino · Mauro Papotti · Francesca Pietribiasi
Gianni Bussolati

Diagnostic cytological features of neuroendocrine differentiated carcinoma of the breast

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Abstract Neuroendocrine (NE) features characterize a minority of carcinomas of the breast corresponding to definite subtypes, which cover a wide spectrum of differentiation. Breast metastases from NE tumours of gastrointestinal origin are not rare, and to determine whether NE carcinomas in the breast could be differentiated from other tumours on fine needle aspiration (FNA) we analysed the cytological features of 13 primary NE breast carcinomas of different types (7 carcinoid-like, 5 mucinous and 1 solid spindle cell). Smears of carcinoid-like carcinomas showed specific features that made it possible to differentiate them from other primary tumours, but not from breast metastases of NE carcinomas. These features were: cell clusters with rigid borders, single cells with a plasmacytoid appearance and peripheral cytoplasmic granules evident on Giemsa staining and immunoreactive for chromogranin A. In mucinous NE carcinomas such granules were less apparent, and the cytological features could have been mistaken for those of fibroadenomas, as in the case of non-NE mucinous carcinomas. The solid spindle cell type showed noncohesive fusiform cells and moderate nuclear pleomorphism, a pattern similar to that of atypical carcinoids of the lung.

Key words FNA cytology · Neuroendocrine breast carcinoma

Introduction

Neuroendocrine (NE) differentiation can occur in specific subtypes of breast carcinomas [18, 21]. The majority of these are argyrophilic with the Grimelius procedure and positive for NE immunocytochemical markers,

such as chromogranins [3, 21]. A common morphological hallmark of NE carcinomas, regardless of the primary site but with the exception of the rare small undifferentiated cell and spindle cell types, is a low grade of cytological atypia [16, 21]. This feature may be misleading on cytological preparations, and some authors suggest that argyrophilia, although nonspecific, could be used as a marker for malignancy in suggestive cytological preparations [22].

A nodule of NE carcinoma inside the breast may also be the presenting clinical finding of metastatic carcinoid [11, 24]; in a large series of reported cases of metastases to the breast up to 3% were from gastrointestinal carcinoids [20]. Surprisingly, these rare tumours give rise to breast metastases more frequently than other, more common tumours, such as colo-rectal adenocarcinoma. In a preoperative diagnosis of breast tumour, both primary and metastatic NE carcinoma should be considered.

The fine needle aspiration (FNA) cytological findings of NE carcinomas in the breast have previously been described in single case reports dealing with five primary [2, 8, 12, 15, 23] and two metastatic tumours [1, 17]. We assessed the FNA cytological features of 13 cases of primary NE differentiated carcinomas of the breast using different staining methods and related them to the histological and immunocytochemical findings. In addition, we considered the cytological differential diagnosis against non-NE low-grade carcinomas and fibroadenomas.

Materials and methods

A series of 105 carcinomas of the breast showing NE differentiation was collected from the files of our pathology departments. In all cases the histological diagnosis was confirmed by means of specific NE markers, such as chromogranins and synaptophysin [21]. In 15 cases a preoperative FNA had been performed. Two cases of mixed mucoid (80%) and solid carcinoma (20%) were nonetheless excluded, being positive for chromogranins in the solid component only, which was almost absent in the cytological smears. The remaining 13 cases with FNA form the basis of the present report (Table 1). The histological diagnosis in these cases

A. Sapino · M. Papotti · G. Bussolati (✉)
Department of Biomedical Sciences and Oncology,
University of Turin, Via Santena 7, I-10126 Torino, Italy
Tel.: +39-11-6706505, Fax: +39-11-6635267

F. Pietribiasi
Department of Pathology, Santa Croce Hospital, Moncalieri,
Torino, Italy

Table 1 Clinical and pathological data in 13 cases of NE differentiated breast carcinoma (*ICC/Cg-A* immunocytochemistry [expressed as % of chromogranin A positive cells], *mts* metastases; *LN* lymph nodes)

Case no.	Age (years)	Size (cm)	Spreading	Histological type	ICC/Cg-A
1	65	1.6	Liver mts	Carcinoid-like	90
2	70	1.7	Axillary LN	Carcinoid-like	90
3	83	3	–	Carcinoid-like	90
4	71	4	Axillary LN	Carcinoid-like	90
5	64	1.4	–	Carcinoid-like	50
6	66	2.3	Axillary LN	Carcinoid-like	90
7	88	3.1	–	Carcinoid-like	90
8	70	2.5	–	Mucinous	50
9	65	2	–	Mucinous/ spindle	90
10	77	1.1	–	Mucinous	50
11	73	1	–	Mucinous + solid	80
12	53	3.5	–	Mucinous/signet	80
13	68	1.8	Axillary LN	Solid spindle cell	70

Table 2 Comparison of FNA cytological features between NE differentiated and non-NE low-grade carcinomas and fibroadenomas of the breast (*italic type* indicates useful differentiation features)

	NE carcinomas			Non-NE carcinomas			
	Carcinoid-like (7 cases)	Solid spindle cell (1 case)	Mucinous (5 cases)	Mucinous (2 cases)	Ductal (2 cases)	Lobular (2 cases)	Fibro-adenoma (2 cases)
Background	Clean	Necrotic	<i>Colloid</i>	<i>Colloid</i>	Clean	Clean	<i>Clean or mixoid</i>
Cellularity	High	High	High	High	Variable	Variable	High
Cell clusters	Numerous cohesive	Rare and small	Numerous, loose	Numerous loose	Cohesive	Absent	Numerous cohesive
Single cells	Numerous	Numerous	Numerous	Numerous	Rare	Numerous	Rare
Cell shape	<i>Plasmacytoid in single cells, rigid and polygonal in cell clusters</i>	<i>Spindle</i>	<i>Plasmacytoid and elongated</i>	<i>Plasmacytoid and elongated</i>	Roundish or cuboidal	Roundish or cuboidal	Roundish or cuboidal
Cytoplasmic granules	<i>Peripheral</i>	<i>Diffuse</i>	<i>Diffuse</i>	Absent	Absent	Absent	Absent
Nuclear location	<i>Eccentric</i>	<i>Eccentric</i>	<i>Central or eccentric</i>	Central or eccentric	Central	Central or eccentric	Central
Nuclear pleomorphism	Low	High	Low	Low	Low	Low	Low
Chromatin	Finely granular or salt and pepper	Coarsely granular	Finely granular	Finely granular	Finely granular	Finely granular	Finely granular
Nucleoli	Small	Small	Small	Small	Small	Small	Absent
Bare neoplastic nuclei	Absent	Absent	<i>Present</i>	<i>Present</i>	Absent	Rarely present	Absent
Bare bipolar nuclei ^a	Absent	Absent	Absent	Absent	Absent	Absent	<i>Present</i>

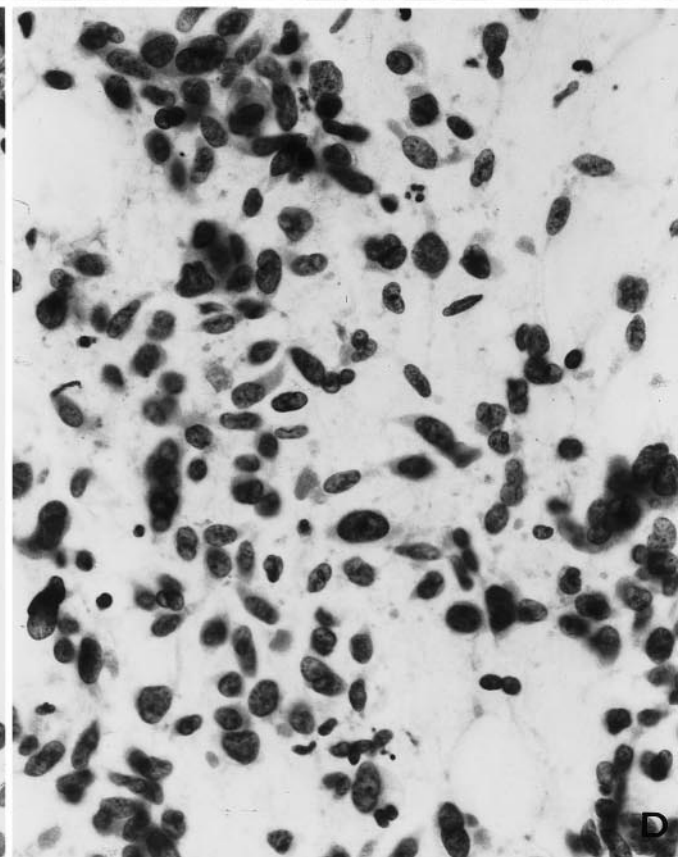
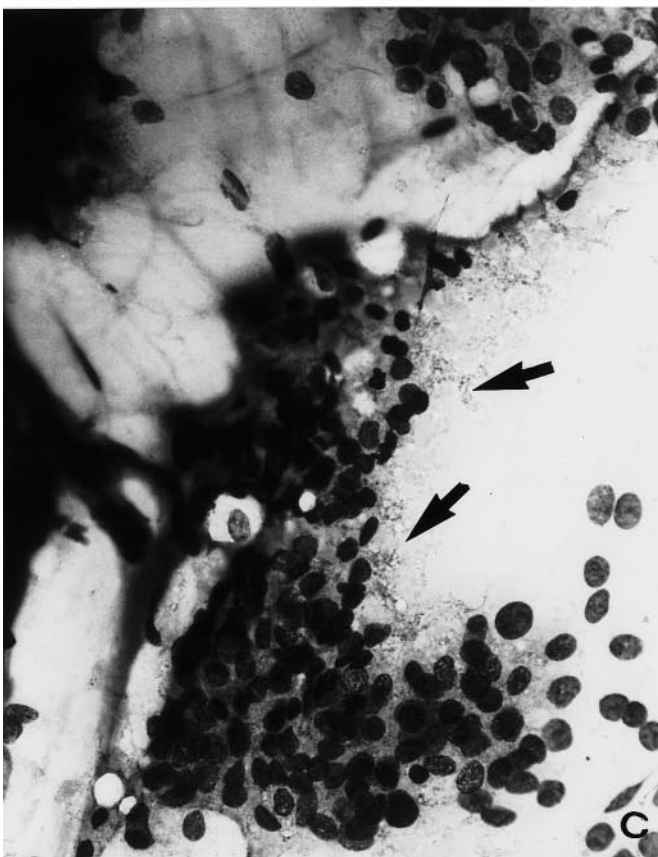
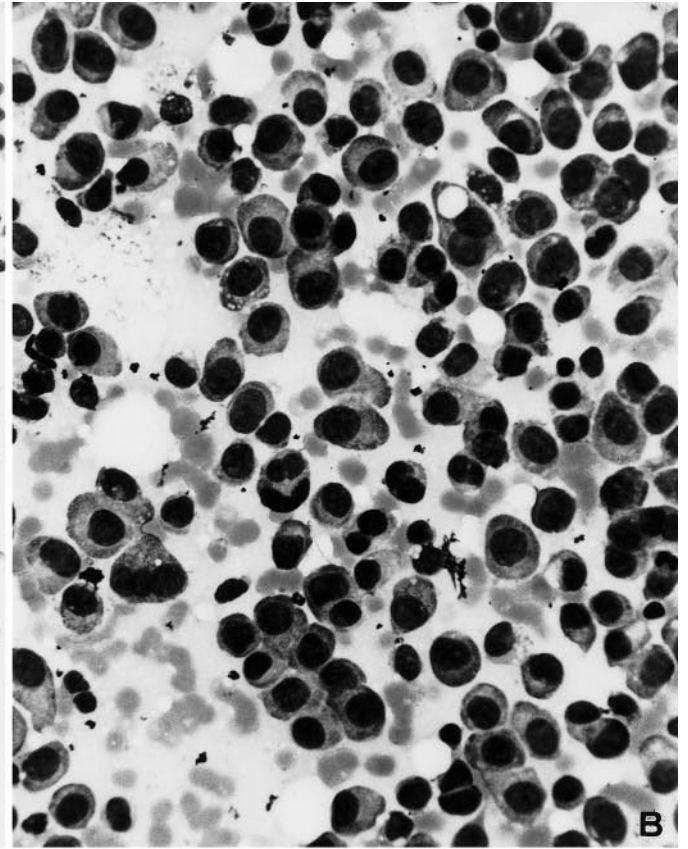
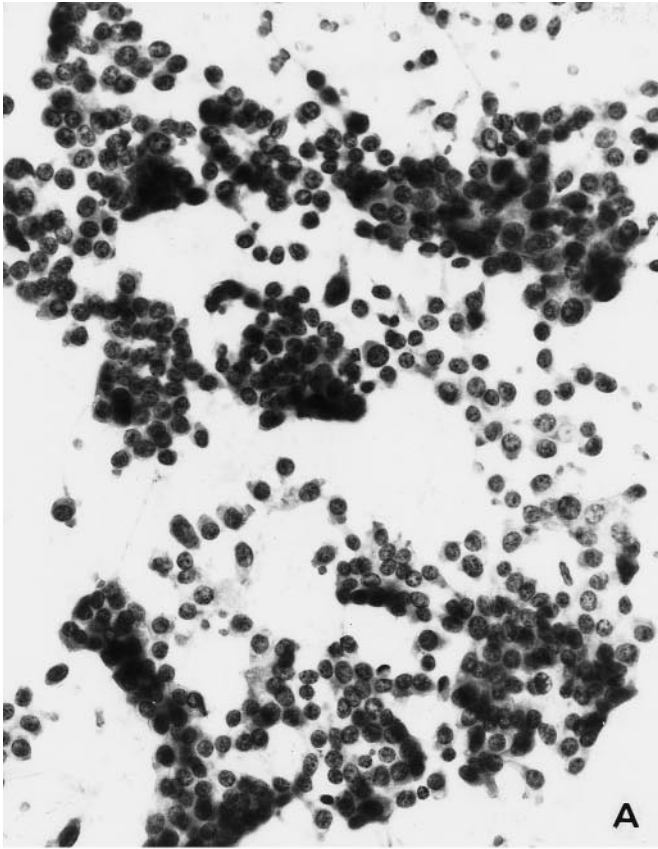
^a Of myoepithelial origin

was cohesive carcinoid-like carcinoma [21] in 7 cases and mucinous carcinoma [4] in 5 cases (1 of these cases had focal signet ring cells [10], 1 had spindle cells [2, 19] and still another case had peripheral foci of in situ solid NE carcinoma). One case was solid with spindle cells and foci of necrosis, a pattern altogether similar to so-called atypical carcinoid of the lung [21]. In 2 cases, FNA of liver and of lymph node metastases were also available.

All the above cases were immunoreactive for chromogranin A in 50–90% of cells.

Immediately after FNA of breast nodules, several smears were prepared for cytological analysis. One slide was fixed in methanol

Fig. 1A–D Fine-needle aspirate (FNA) smears of *carcinoid-like* NE breast carcinomas. **A** At low power, a highly cellular smear shows cells arranged in cohesive clusters and dispersed in a clean background. H&E, ×200 **B** In one case, the majority of the cells have a plasmacytoid appearance and are singly dispersed. Giemsa, ×450 **C** FNA smear of *mucinous* NE breast carcinoma. Clusters of carcinoma cells are loosely dispersed in a colloid-like substance. The cells show a finely granular cytoplasm (*arrows*). Giemsa, ×400 **D** FNA smear of solid spindle cell NE breast carcinoma shows numerous noncohesive fusiform cells in a necrotic background. Nuclei are moderately pleomorphic with small nucleoli. Papanicolaou, ×400



and stained with rapid haematoxylin and eosin (H&E) to check sample adequacy. The other slides were either air dried and stained with Giemsa or fixed in 95% (vol/vol) ethanol and stained with Papanicolaou's method.

All smears of the 13 cases of NE carcinoma entered in this study were reviewed to evaluate the following cytological features: background, cellularity, cell aggregation, presence of neoplastic bare nuclei, cell shape, nuclear morphology and location, chromatin characteristics, nucleolar morphology. The same morphological features were evaluated in FNA of 8 control cases of low-grade ductal carcinoma, lobular carcinoma, non-NE mucinous carcinoma and fibroadenoma.

To assess the NE nature of the cells one H&E-stained smear of each case was unmounted, rehydrated in distilled H₂O, rinsed in PBS and incubated for 30 min at room temperature with anti-chromogranin A monoclonal antibody LK2H10 (diluted 1:50; BioGenex, San Ramon, Calif.). The reaction was revealed by incubation with the LSAB-2 streptavidin peroxidase system (Dakopatts, Glostrup, Denmark).

Results

The main cytological features are summarized in Table 2.

At low power, cellularity was seen to be high in all smears. In the carcinoid-like variant the cells were either arranged in cohesive clusters of various sizes or dispersed singly against a clean background (Fig. 1A). In 1 case, the single cells were predominant (Fig. 1B). In the

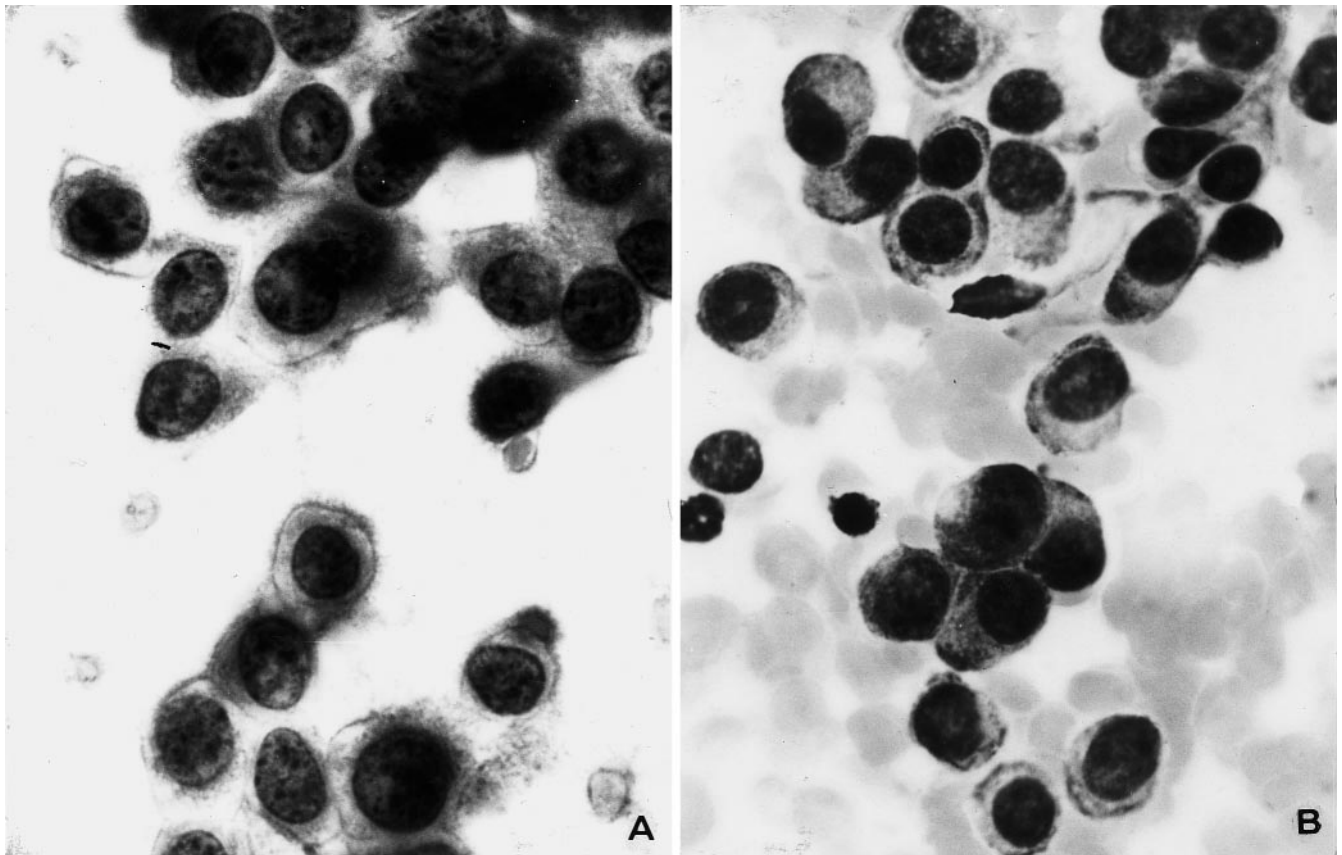
mucinous type, loose or cohesive aggregates of cells were dispersed in a colloid-like substance, which stained bluish or light violet with Giemsa (Fig. 1C) and H&E, and a barely visible light blue-green with Papanicolaou's procedure. Smears of the solid spindle cell NE carcinoma showed numerous noncohesive fusiform cells against a necrotic background (Fig. 1D).

At high power, in the carcinoid-like variant the clusters were formed by polygonal cells arranged in a "cobblestone" pattern, which was particularly evident in H&E-stained slides (Fig. 2A). Single cells had a plasmacytoid appearance and eccentrically located nuclei. These were roundish and finely granular owing to the "salt and pepper" distribution of the chromatin. Rare cells were binucleated. One or two inconspicuous nucleoli were evident. The Giemsa staining marked bluish granules arranged as a rim at the cell borders (Fig. 2B) or as a polar cap in single plasmacytoid cells (Fig. 3A). Chromogranin A immunoreactivity had the same pattern of distribution as the cytoplasmic granules (Fig. 3B).

The smears of liver and lymph node metastases showed the same cytological features.

In the mucinous NE carcinoma the cells were larger than in the solid type; the cytoplasm was pale on H&E and Papanicolaou staining, and finely granular on Giemsa staining (Fig. 1C). Chromogranin A generally had weak and diffuse cytoplasmic reactivity, but rare cells were strongly positive. The nuclei were roundish with slight variation in size and were eccentrically or centrally placed. Bare neoplastic nuclei were present. The signet ring cells, focally present in 1 case of mucinous carcino-

Fig. 2A, B FNA smears of *carcinoid*-like NE carcinomas. **A** At high power the clusters are formed by polygonal cells with defined borders arranged in a "cobblestone" pattern. H&E, $\times 1000$ **B** Giemsa staining outlines numerous cytoplasmic granules, particularly evident at the cell border. $\times 1000$



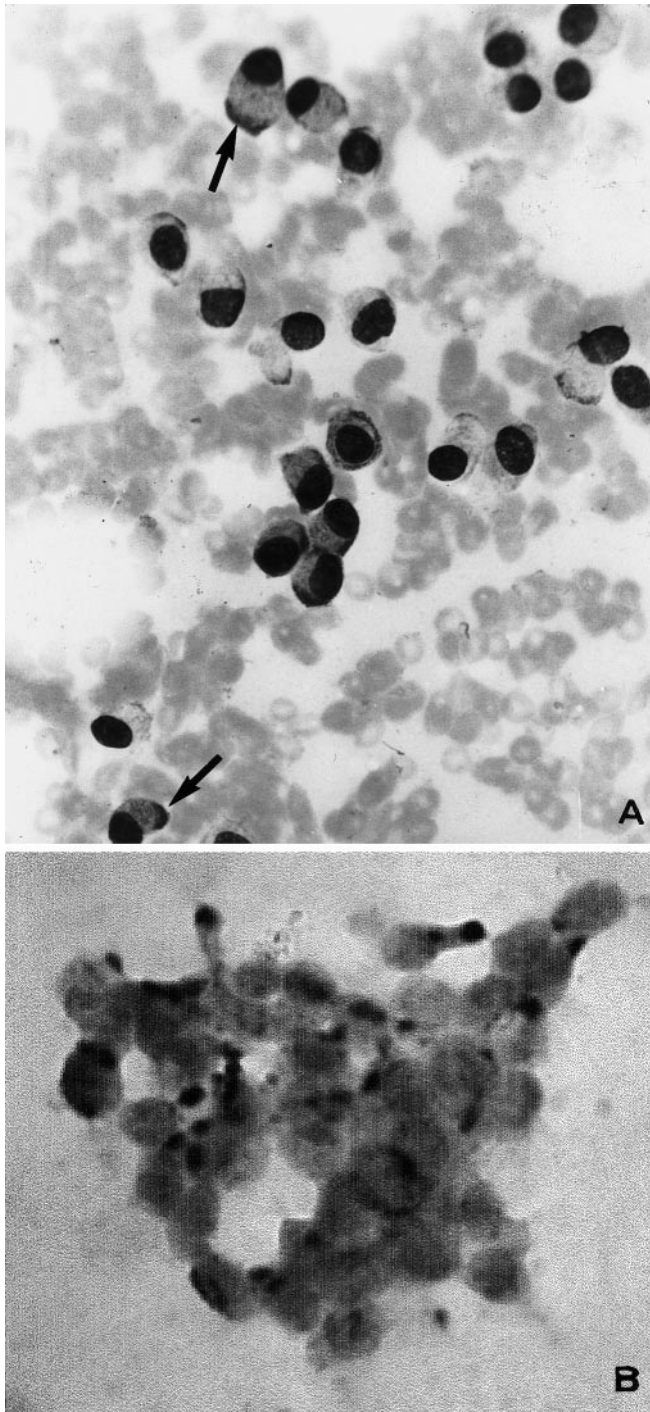


Fig. 3 **A** Giemsa staining of a smear of *carcinoid*-like NE carcinoma. The single cells show a plasmacytoid appearance and a cap of bluish granules at a cytoplasmic pole (arrows). $\times 500$ **B** Immunocytochemical staining for chromogranin A outlines the NE granules, which have the same cytoplasmic distribution as the Giemsa-stained granules. $\times 500$

ma, had more pleomorphic nuclei, prominent nucleoli and intracytoplasmic vacuoles abutting the nuclei. The spindle cell variant of mucinous carcinoma showed sheets of uniform elongated cells with abundant vacuolated cytoplasm. Intracytoplasmic granules positive for chromogranin A were present.

The solid spindle cell variant showed elongated cells with scanty cytoplasm, moderate nuclear pleomorphism and small nucleoli (Fig. 1D). A granular positivity for chromogranin A was seen within the cytoplasm.

Discussion

We report the FNA cytological findings recorded in a series of primary NE differentiated carcinomas of the breast. Only the smears of the carcinoid-like variant showed specific features that allowed us differentiate them from other primary tumours, and they did not make differentiation from breast metastases of NE carcinomas possible. The features, previously described in single case reports of primary or metastatic "carcinoid" breast tumours [1, 2, 8, 12, 15, 17, 23], are high cellularity, bland cytology, and a plasmacytoid cell appearance resulting from eccentrically located roundish nuclei. In our study, in addition to these features, we found that the carcinoid-like variant showed clustered cells with defined cytoplasmic borders on H&E and peripherally located intracytoplasmic granules on Giemsa staining. The same staining marked a cap of granules at one pole of single plasmacytoid-like cells. Other authors have reported that 60% of cells in argyrophilic carcinoma show prominent eosinophilic granularity with Diff-Quick staining and not with Papanicolaou staining [12]. Diffuse eosinophilic cytoplasmic granules, which are easily visible with H&E, are found in various breast tumours, such as apocrine differentiated carcinoma and granular cell tumour. However, these lesions show higher cytological pleomorphism and are positive for other specific immunological markers than chromogranins [25]. In our series of low-grade NE carcinoma of the breast, the presence of peripheral cytoplasmic granules positive for chromogranin A can be considered specific to such tumours and can be used to differentiate them from non-NE low-grade carcinomas of ductal or lobular type. In 1 case of carcinoid-like NE tumour the FNA cytology gave rise to smears with predominantly single cells showing a plasmacytoid appearance, which led to differential diagnosis against plasmacytoma. However, the absence of the typical cartwheel nuclei and the presence of chromogranin A-positive cytoplasmic granules suggested the diagnosis of NE tumour.

Mucinous NE carcinomas of the breast can be classified into a non-NE variety (type A), and an NE differentiated variety (type B), according to Capella et al. [4]. The FNA cytological features of the NE variety are almost identical to those of the non-NE variety [7]. The presence of colloid-like background is essential for the diagnosis of mucinous carcinoma. In our experience, this material is better outlined in air-dried smears stained by Giemsa, as also noted by Duane [7] using Diff-Quick staining. In our series of NE mucinous carcinomas, the presence of granules was not as evident as in the solid variant, probably because they were scattered in the mucus. In these cases, as also previously reported for non-NE colloid carcinomas, fibroadenoma with myxoid stroma has to be considered in the differential diagnosis [10]. In both tumours, clusters of regular cells may be dispersed in a mu-

coid background, but in smears of fibroadenoma the presence of bipolar bare nuclei of myoepithelial origin, which are absent in mucinous carcinoma, is quite specific.

The solid spindle cell variant of NE carcinoma of the breast, defined by Papotti et al. [21] as type E, cannot be reliably recognized by cytological methods without the use of immunohistochemistry. In fact, on cytological preparations, necrosis, fusiform cells and moderate nuclear pleomorphism are suggestive of a primary high-grade spindle cell carcinoma, such as metaplastic carcinoma. However, the absence of prominent nucleoli would exclude such a diagnosis. A differential diagnosis from a metastasis of spindle cell NE carcinoma of the lung (so-called atypical carcinoid) also has to be considered [5]. In their book on FNA cytology of lung and breast, Feldman and Covell [9] described the cytological features of spindle cell carcinoid of the lung, which overlapped with those of our case in the breast. (Compare our Fig. 1D with plate 46, p. 199 in [9]).

Although the purpose of FNA of breast lesions is to select malignant cases and cases of suspected malignancy for surgical procedures, in dealings with NE tumours the possibility of metastases of NE tumour from other sites has to be considered [11, 14, 20, 24]. Morphology alone is not a reliable criterion for distinguishing a metastatic breast NE carcinoma from a primary when both have similar cytological features [16]. In addition, carcinoids can metastasize to the breast as an isolated breast mass, and conversely primary breast NE carcinomas can present as bilateral lesions [6, 11].

In conclusion, a diagnosis of NE differentiated carcinoma of the breast is possible or can at least be suspected in FNA cytological material. At present, such a diagnosis does not involve a different treatment than for other types of breast carcinomas of the same stage. However, recent evidence suggests that chromogranin production in NE breast carcinomas might have clinical and genetic implications, owing to the biochemical analogy between granins and BRCA1 protein [13]. Finally, the cytological features of NE carcinomas of the breast overlapped with those of NE carcinomas in other sites, and their identification by means of a preoperative FNA procedure could allow an accurate clinical study of patients and make it possible to avoid unnecessary radical treatment in the case of metastatic tumours.

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