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Case report

Primary oat cell (neuroendocrine) carcinoma of the breast

Report of four cases

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Summary. Four cases of oat cell (neuroendocrine) carcinoma of the breast are reported. Three patients died within 15 months of the diagnosis and the fourth patient is alive after 44 months. Histochemical, ultrastructural and mRNA markers of endocrine differentiation were present in three cases. These tumours show histological similarities to breast metastases of bronchial oat cell carcinoma, but a distinguishing feature is the presence of in situ ductal lesions. It appears that the breast is a further site which has to be added to the long list of extrapulmonary oat cell carcinomas.

Key words: Breast cancer – Oat cell carcinoma – Neuroendocrine – In situ hybridization

Introduction

Neuroendocrine (NE) differentiation in breast carcinoma was originally described by Cubilla and Woodruff (1977) and further attention was drawn to it by Azzopardi et al. (1982) and Nesland et al. (1988). These tumours show a broad morphological spectrum including mucoid carcinomas (Capella et al. 1980) and atypical carcinoids (Papotti et al. 1989). Only two cases of primary oat cell (NE) carcinoma have been recorded in the literature (Wade et al. 1983; Jundt et al. 1984), one of which occurred in a male patient.

We report four additional cases of primary oat cell carcinoma (OC) in the breast and emphasize their diagnostic features.

Materials and methods

Four cases of primary OC of the breast from a series of 77 NE differentiated breast carcinomas were selected from the consultation files of two of us (G.B. and V.E.). Histological material for further investigation was retrieved and complete clinical and follow-up information was obtained. Two cases (cases 2 and 3) had been included in a previous study of a large series of NE differentiated breast carcinomas (Papotti et al. 1989).

Tissues were fixed in Bouin's solution or 10% formaldehyde and embedded in paraffin. Haematoxylin and eosin (H&E) stained sections were obtained and serial sections were immunostained with antibodies to general NE markers and to bombesin and serotonin. Smooth muscle actin immunostaining was also used to reveal myoepithelial cells (Table 1). The immunoperoxidase procedure with the avidin biotin complex according to Hsu et al. (1981) was employed. Endogenous peroxidase activity was inhibited according to Heyderman and Neville (1977).

Serial sections were also collected on polylysine-coated slides and processed for in situ hybridization. Chromogranin(s) mRNA expression was evaluated using oligonucleotides specific to chromogranin A (CgA) and B (CgB), labelled with a digoxigenin UTP tail, revealed with a non-radioactive DNA labelling and detection kit (Boehringer, Mannheim, FRG), as described by Pagani et al. (1991).

Ultrastructure was investigated in two cases. Glutaraldehyde-fixed samples were processed by routine methods.

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Case reports

Case 1

The patient, a 64-year-old lady, presented in May 1987 with a 3-month history of a hard nodule in the upper inner quadrant of the right breast. Nodulectomy was performed and a 2-cm tumour showing irregular margins was removed. No residual tumour was found 1 month later when the patient underwent radical mastectomy with axillary dissection of ten lymph nodes which did not show any evidence of metastasis. Chest CT scan performed at the time of surgery and 18 months later did not reveal any lung involvement. The tumour was oestrogen receptor negative. No further treatment was given to the patient, who is currently free of disease 44 months after operation (Table 2).

Case 2

The patient, a 41-year-old lady, presented in February 1987 with a short history of an enlarging nodule in the upper outer quadrant of the left breast. Past history was unremarkable. Mammography

Table 1. Antibodies employed for immunoperoxidase

Marker	Type of reagent	Source	Dilution
Chromogranin A	M (LK2H10)	Dr. R.V. Lloyd (Ann Arbor, Mich., USA)	1/100
Chromogranin B	P	Dr. H. Winkler (Innsbruck, Austria)	1/500
	M (B11)	Dr. R. Buffa (Varese, Italy)	1/1000
Secretogranin II	M	Dr. M. Pelagi (Milan, Italy)	1/1000
Synaptophysin	P	Dr. F. Navone (Milan, Italy)	1/200
NSE	M	Sambio (Am Uden, The Netherlands)	1/50
GRP	P	Milab (Knivsta, Sweden)	1/500
Serotonin	P	Sambio	1/1000
Leu7	M	Becton Dickinson (Milan, Italy)	1/200
Actin	M (asm)	Dr. G. Gabbiani (Geneva, Switzerland)	1/1000
HMFG2	M	Serotec (Oxford, UK)	1/100

NSE, Neuron-specific enolase; GRP, gastrin-releasing peptide; M, Monoclonal; P, polyclonal; HMFG2, human milk fat globule membrane

Table 2. Reported cases of oat cell (neuroendocrine) carcinoma of the breast

Cases	Sex/ age	Site	Size (cm)	In situ lesion	Vascular invasion	LN	Treatment	Outcome
Wade et al. (1983)	F/52	ROQ	10	−	+	25/25	RM/Ch	DOD 9 months
Jundt et al. (1984)	M/52	RSA	NS	−	+	+	RT/Ch	DOD 14 months
Present case 1	F/64	RUIQ	2	+	+	0/10	RM	NED 44 months
Present case 2	F/41	LUOQ	3.5	+	+	7/14	RM/RT	DOD 15 months
Present case 3	F/41	LUOQ	3	+	+	3/18	RM/Ch	DOD 14 months
Present case 4	F/69	LUQ	5	+	+	3/10	RM/H	DOC 9 months

LN, Axillary lymph node metastases; NED, no evidence of disease; DOD, died of disease; DOC, died of other cause; NS, not stated; R, right; L, left; UIQ, upper inner quadrant; RM, radical mastectomy; UOQ, upper outer quadrant; RT, radiotherapy; Ch, chemotherapy; UQ, upper quadrants; OQ, outer quadrants; SA, subareolar; H, hormonal treatment

disclosed a 3.5-cm mass with irregular borders. Radical mastectomy and axillary lymph node dissection was performed. Chest CT scan performed at the time of operation did not reveal any lung involvement. The tumour was of firm consistency and showed infiltrating margins. No residual tumour was found in the remaining breast. Seven of 14 axillary lymph nodes were involved by metastatic spread. No oestrogen receptor positivity was found. The patient received additional radiotherapy. After a year apparently disease-free, metastases spread to several organs and the patient died 15 months after surgery. An autopsy was not performed.

Case 3

The patient, a 50-year-old lady, presented in November 1987 with a 1-month history of a nodule in the left breast. Past history was unremarkable. Mammography and ultrasonographic scan revealed a nodule showing irregular borders in the upper outer quadrant. This measured 3 cm in greatest dimension. A fine-needle aspiration indicated malignancy. Radical mastectomy was performed with axillary lymph node dissection. A 3×2 cm, firm greyish mass with infiltrating margins was found. The remaining breast showed fibrofatty tissue and no residual tumour was present. Three of 18 axillary lymph nodes contained metastatic deposits. Oestrogen and progesterone receptor analysis gave negative results. Chest CT scan at the time of operation did not show any lung involvement. The patient underwent a cycle of streptozotocin which could not be completed due to the appearance of side effects. In March 1988, ultrasonography and total body CT scan revealed two metastatic foci in the liver and a 2-cm hypodense lesion in the left cerebellar hemisphere. In April 1988 a cycle of chemotherapy with CMF (cyclophosphamide, methothrexate and fluorouracyl) was administered together with brain irradiation (40 Gy). In July 1988 a recurrence near the surgical scar appeared, and was excised. Chest radiography performed at that time excluded any lung involvement, while CT scan showed additional cerebellar lesions. From August 1988 the patient, who refused any further therapy, developed right hemiparesis, multiple subcutaneous and pulmonary metastases (January 1989). She died shortly thereafter. An autopsy was not performed.

Case 4

The patient, a 68-year-old lady, presented in December 1987 with a 4-month history of a left breast mass. Past history was unremarkable. In July 1987 the patient had been admitted to another hospital with right hemiparesis. An acute cerebrovascular episode was diagnosed. The CT scan was not available for review. On physical examination a mass in the upper quadrants was found and a modified radical mastectomy with axillary node dissection was performed. A firm, greyish, 5-cm tumour was found with irregular borders. Three of ten axillary lymph nodes were involved by metastases, but no areas of tumour were found in other zones of the breast. Oestrogen and progesterone receptors were positive and additional hormonal treatment (tamoxifen) was administered. Repeated CT scans did not reveal any lung involvement. The patient died 9 months after operation of a presumptive cerebral haemorrhage. Autopsy was not requested.

Results

The four tumours were composed of small round to ovoid cells with hyperchromatic nuclei and scant cytoplasm. The neoplastic cells formed clusters, large sheets or trabeculae, separated by thick fibrous bundles (Fig. 1). In case 4 the small cell component constituted about 40% of the neoplastic population, while the remaining tumour consisted of spindled or round elements with eosinophilic cytoplasm. These formed alveolar structures reminiscent of the alveolar variant of invasive lobular carcinoma as defined by Martinez and Azzopardi (1979). All the tumours had extensive areas of necrosis and a high mitotic count (average 9.8×10 HPF, range 3–19). Vascular invasion at the periphery of the neoplastic nodule was prominent in all cases (Fig. 2). The metastatic deposits in lymph nodes in cases 2, 3 and 4 showed similar features to their respective primaries.

Small ducts filled with neoplastic cells were found in all cases (Fig. 3) either in the centre or close to the margins of the lesions. The neoplastic cells present within the ducts showed similar morphological features to the neoplastic elements of the invasive counterpart. Using actin antibodies, a discontinuous layer of flattened myoepithelial cells was recognizable at the periphery of the involved ducts (Fig. 3). In case 3 neoplastic cells were also present along the walls of large ducts, in a pagetoid fashion displacing columnar epithelium towards the lumen.

Grimelius silver impregnation was positive in cases 3 and 4, varying from 50 to 80% of the total neoplastic population. The same two cases were reactive for CgA (case 3) and CgB (case 4). No immunoreactivity was observed for secretogranin II (chromogranin C). Synaptophysin, serotonin and Leu 7 were positive in two cases each. Neuron-specific enolase (NSE) and bombesin/gastrin releasing peptide (GRP) reacted in three cases each (see Table 3).

In situ hybridization showed CgA mRNA expression in the majority of neoplastic cells in case 3 (Fig. 4) and CgB mRNA expression in some tumour cells of case 4. No signal was present in the other tumours.

Ultrastructural electron microscopic investigation (in cases 2 and 3) showed dense core neurosecretory granules present in the cytoplasm of tumour cells. In case 2, the granules were small (99 nm), dense and roundish; in case 3 some tumour cells showed relatively large (227 nm) granules, while small (147 nm) dense granules were present in the cytoplasm of other cells. No clear vesicles of the synaptic type, and no mucinous or amphicrine cells were found.

Discussion

The four cases reported here show structural (trabecular), cytological (spindle to lymphocyte-like elements) and nuclear (hyperchromatic, without nucleoli) features

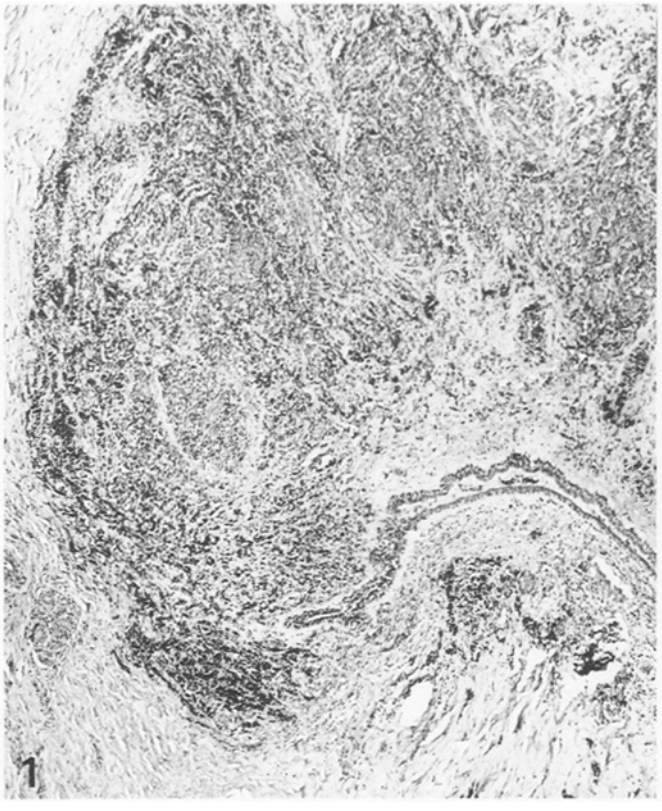

Fig. 1. Case 3. Scanning magnification showing proliferation of small cells in clusters within fibrous stroma and around a large duct containing neoplastic cells. H&E, ×40

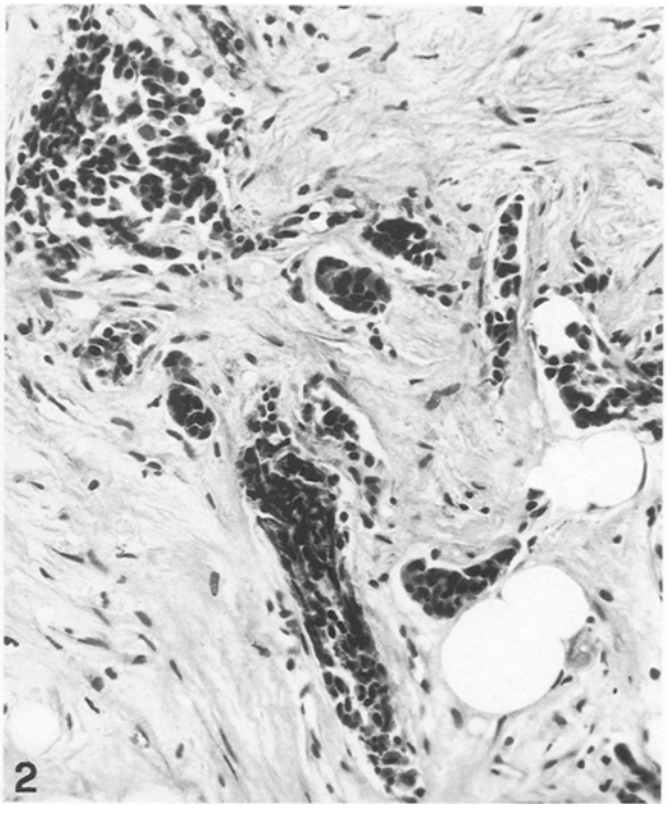

Fig. 2. Case 4. Invasive component of oat cell carcinoma. Fibrofatty stroma is infiltrated by cords and clusters of small cells with dense oval nuclei. Vascular invasion is also prominent. H&E, ×250

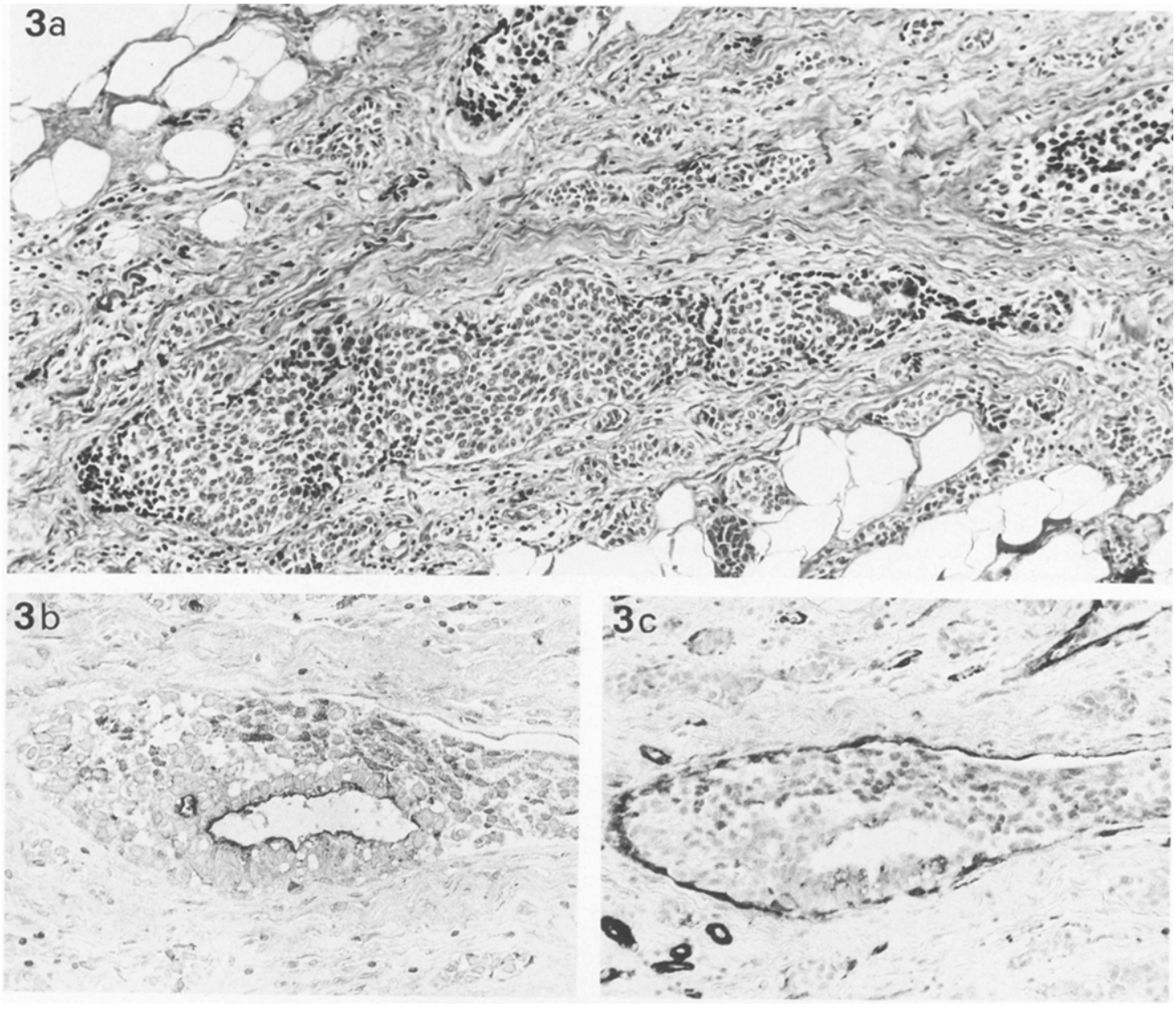

Fig. 3a–c. Case 1. In situ lesions in an oat cell carcinoma. **a** A duct is filled by small neoplastic cells which dilate the duct and displace secretory epithelium towards the centre of the lumen. Residual secretory epithelial cells are well outlined by HMFG2 immunostatining (**b**). The myoepithelial cell layer, easily revealed by actin immunostaining, is still present at the periphery of the duct (**c**). **a** H&E, ×180; **b**, **c** immunoperoxidase with nuclear haemalum counterstain, ×250

Table 3. Immunohistochemical findings in four oat cell carcinomas of the breast

Case no.	Grim	CgA	CgB	SgII	NSE	SY	GRP	Leu7	5HT
1	–	–	–	–	–	–	–	5[a]	–
2	–	–	–	–	70	–	10	10	–
3	80	60	–	–	10	10	10	–	5
4	50	–	50	–	30	90	40	–	20

[a] Percentage of positive neoplastic cells

Grim, Grimelius silver impregnation; CgA, chromogranin A; CgB, chromogranin B, SgII, secretogranin II (chromogranin C); NSE, neuron-specific-enolase; SY, synaptophysin; GRP, gastrin-releasing peptide/bombesin; 5HT, serotonin

consistent with the criteria described by Azzopardi (1959) for OC. Ultrastructurally, these cases showed cytoplasmic endocrine-like granules, as seen by Hattori et al. (1972) in OC of the lung. Additional elements consistent with NE differentiation were positive immunoreaction for both NSE and GRP (cases 2, 3 and 4), serotonin and synaptophysin (cases 3 and 4) and Leu 7 (cases 1 and 2). CgA and CgB were demonstrated in cases 3 and 4 respectively, at both protein and gene levels by immunohistochemistry and in situ hybridization. Case 1, despite being negative with NE cell markers (except for a minor positivity with Leu 7), has been included because of the histological similarity with the other cases.

CgA has been localized immunologically in OC by Weiler et al. (1988) and synaptophysin by Kayser et al. (1988). Chromogranin gene expression has been recently

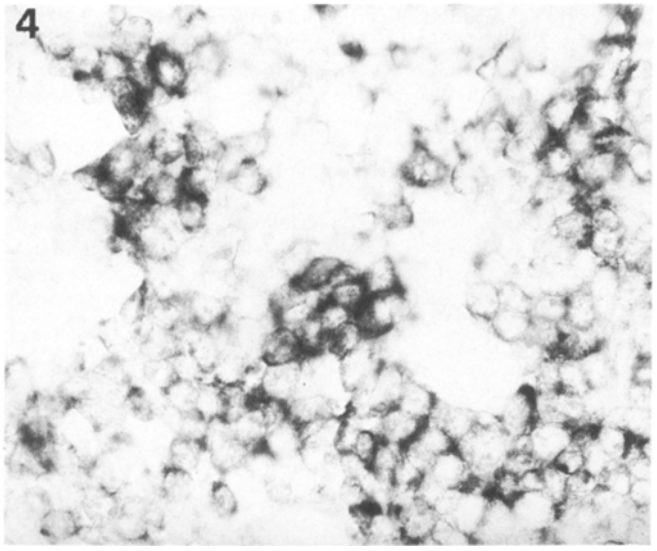

Fig. 4. Case 3. In situ hybridization shows several neoplastic cells strongly expressing chromogranin A mRNA in the cytoplasm. × 500

demonstrated by in situ hybridization in two cases of primary OC of thyroid (Eusebi et al. 1990).

In cases 2 and 3 the tumours showed very aggressive behaviour and the patients died shortly after surgery, while case 4 died for causes apparently unrelated to the breast tumour. In case 1 the patient was in good health 44 months after breast removal. In this patient, tumour size was the smallest and no lymph node metastases were evident at the time of surgery.

Cases histologically similar to the present lesions have been reported previously in two instances by Wade et al. (1983) and by Jundt et al. (1984). However, in neither of the previous reports were in situ lesions detected and our present observation and description appear original.

In the present cases the possibility of a metastasis from a bronchial OC was taken into consideration since it is well known that lung is the most common site of origin of secondary deposits to the breast (Kelly et al. 1988). In all the cases reported here extensive radiological investigations led to the exclusion of sites of origin other than breast. In addition, clear-cut in situ carcinomatous changes were present in all cases, showing cytological details identical to the invasive component. This latter is probably the single feature which confidently indicates the primary nature of a tumour in breast. Therefore it seems that breast has to be added to the long list of organs where primary OC can originate (Eusebi et al. 1978, 1990; Capella et al. 1984).

Since the description of Cubilla and Woodruff (1977) several papers claiming to prove or deny the existence of breast carcinomas with endocrine differentiation have been published. (Fisher et al. 1979; Taxy et al. 1981; Azzopardi et al. 1982; Clayton et al. 1982; Nesland et al. 1986; Bussolati et al. 1987). Recently Papotti et al. (1989), Capella et al. (1990) and Pagani et al. (1990) gave conclusive evidence of the existence of NE differentiated breast carcinomas based on immunohistochemical, ultrastructural and hybridization analyses. Papotti et al. (1989) also codified seven histological patterns of this variety of breast carcinoma, which also includes cases of OC.

In conclusion, our findings indicate that OC can occur primarily in the breast and represents an aggressive but not invariably lethal variant of breast carcinoma. Differential diagnosis from metastatic OC is mandatory and is feasible on histological grounds.

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References

Azzopardi JG (1959) Oat-cell carcinoma of the bronchus. J Pathol Bacteriol 78:513–519

Azzopardi JG, Muretto P, Goddeeris P, Eusebi V, Lauwerying JM (1982) "Carcinoid" tumours of the breast. The morphological spectrum of argyrophilic carcinomas. Histopathology 5:549–569

Bussolati G, Papotti M, Sapino A, Gugliotta P, Ghiringhello B, Azzopardi JG (1987) Endocrine markers in argyrophilic carcinomas of the breast. Am J Surg Pathol 11:248–256

Capella C, Eusebi V, Mann B, Azzopardi JG (1980) Endocrine differentiation in mucoid carcinoma of the breast. Histopathology 4:613–630

Capella C, Eusebi V, Rosai J (1984) Primary oat-cell carcinoma of the kidney. Am J Surg Pathol 8:855–861

Capella C, Usellini L, Papotti M, Macri L, Finzi G, Eusebi V, Bussolati G (1990) Ultrastructural features of neuroendocrine differentiated carcinomas of the breast. Ultrastruct Pathol 14:321–334

Clayton F, Ordonez NG, Sibley RK, Hanssen G (1982) Argyrophilic breast carcinoma. Evidence of lactational differentiation. Am J Surg Pathol 6:323–333

Cubilla AL, Woodruff JM (1977) Primary carcinoid tumor of the breast: a report of eight patients. Am J Surg Pathol 4:283–292

Eusebi V, Betts CM, Giangaspero F (1978) Primary oat-cell carcinoma of the larynx. Virchows Arch [A] 380:349–354

Eusebi V, Damiani S, Riva C, Lloyd RV, Capella C (1990) Calcitonin free oat-cell carcinoma of the thryroid gland. Virchows Arch [A] 417:267–271

Fisher ER, Palekar AS, NSABP collaborators (1979) Solid and mucinous varieties of so-called mammary carcinoid tumors. Am J Clin Pathol 72:909–916

Hattori S, Matsuda M, Tateishi R, Nishihana H, Horai T (1972) Oat-cell carcinoma of the lung. Clinical and morphological studies in relation to its histogenesis. Cancer 30:1011–1024

Heyderman E, Neville AM (1977) A shorter immunoperoxidase of carcino-embryonic-antigen and other cell products. J Clin Pathol 30:138–140

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Jundt G, Schultz A, Heitz PU, Osborn M (1984) Small cell neuroendocrine (oat cell) carcinoma of the male breast. Immunocytochemical and ultrastructural investigations. Virchows Arch [A] 404:213–222

Kayser K, Schmid W, Ebert W, Wiedenmann B (1988) Expression of neuroendocrine markers (neuron-specific-enolase, synaptophysin and bombesin) in carcinoma of the lung. Pathol Res Pract 183:412–417

Kelly C, Henderson D, Corris P (1988) Breast lumps: rare presentation of oat cell carcinoma of lung. J Clin Pathol 41:171–172

Martinez V, Azzopardi JG (1979) Invasive lobular carcinoma of the breast: incidence and variants. Histopathology 3:467–488

Nesland JM, Holm R, Johannessen JV (1986) A study of different markers for neuroendocrine differentiation in breast carcinomas. Pathol Res Pract 181:524–530

Nesland JM, Holm R, Johannessen JV, Gould VE (1988) Neuroendocrine differentiation in breast lesions. Pathol Res Pract 183:214–221

Pagani A, Papotti M, Höfler H, Winkler H, Weiler R, Bussolati G (1990) Chromogranin A and B gene expression in carcinomas of the breast. Am J Pathol 136:319–327

Pagani A, Forni M, Tonini GP, Papotti M, Bussolati G (1991) Expression of members of the chromogranin family in primary neuroblastomas. Diagn Mol Pathol (in press)

Papotti M, Macrì L, Finzi G, Capella C, Eusebi V, Bussolati G (1989) Neuroendocrine differentiation in carcinomas of the breast. A study of 51 cases. Semin Diagn Pathol 6:174–188

Taxy JB, Tischler AS, Insalaco SJ, Battifora H (1981) "Carcinoid" tumor of the breast. A variant of conventional breast cancer? Hum Pathol 12:170–179

Wade PM, Mills SE, Read M, Cloud W, Lambert MJ, Smith RE (1983) Small cell neuroendocrine (oat cell) carcinoma of the breast. Cancer 52:121–125

Weiler R, Fisher-Colbrie R, Schmid KW, Feichtinger H, Bussolati G, Krisch K, Kerl H, O'Connor D, Winkler H (1988) Immunological studies on the occurrence and properties of chromogranin A and B and secretogranin II in endocrine tumors. Am J Surg Pathol 12:877–884