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Olfactory neuroblastoma: Clinical and pathological aspects *

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SUMMARY

Twenty cases of olfactory neuroblastoma were available for clinical and histopathological evaluation. The usefulness of immunohistochemistry in the diagnosis of this tumour was investigated and was best achieved using a panel of monoclonal and polyclonal antibodies, notably neuron-specific enolase, PGP 9.5, S-100 protein, synaptophysin and chromogranin A. This study confirmed that immunohistochemistry is a useful adjunct in cases where conventional histology is equivocal.

Key words: olfactory neuroblastoma, immunohistochemistry, diagnostic pathology

INTRODUCTION

Olfactory neuroblastoma – or 'esthesioneuroepithelioma olfactif' as it was originally termed in 1924 by Berger et al. – is a rare tumour arising from olfactory epithelium. Although olfactory tumours have been produced in the Syrian hamster in response to diethylnitrosamine (Herrold, 1967), no predisposing factors are known in man and unlike neuroblastomas in other sites in the body, it arises in adulthood. Olfactory neuroblastoma can sometimes present difficulties in diagnosis and this study was primarily performed to determine the role of immunohistochemistry in this condition.

MATERIAL AND METHODS

Twenty patients with olfactory neuroblastoma were treated between 1976 and 1990 (Table 1). Sixteen patients had had no previous treatment, two had already undergone lateral rhinotomy, one an external ethmoidectomy and one a transantral ethmoidectomy. Five patients had been treated with radiotherapy prior to referral. Treatment of

Table 1. Clinical features of patients with olfactory neuroblastoma.

patient	age (years)	sex	surgery	radiotherapy	follow-up (months)	outcome
1	20	F	LR	post-operative	180	AEW
2	59	M	LR	post-operative	lost	–
3	45	M	LR	pre-operative	156	AEW
4	17	M	LR	post-operative	10	DOD
5	23	M	none	to primary and neck	lost	–
6	54	M	CF	pre-operative	102	DOD
7	62	M	CF	none	89	AEW
8	57	F	CF	pre-operative	120	DOD
9	26	F	CF	none	27	AEW
10	47	F	CF	none	44	AEW
11	67	F	CF	none	43	AEW
12	61	F	CF	none	39	AEW
13	14	M	CF	pre-operative	31	DICD
14	57	M	CF	none	30	DOD
15	57	F	CF	pre-operative	70	AEW
16	38	M	CF	post-operative	48	AEW
17	37	M	CF	pre-operative	4	AEW
18	47	M	CF	none	28	AEW
19	64	M	CF	none	36	AWR
20	52	F	CF	none	55	AWR

AEW: alive and well; AWR: alive with recurrence; DOD: dead of disease; DICD: dead of intercurrent disease; LR: lateral rhinotomy; CF: craniofacial resection.

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Table 2. List of antibodies applied to sections of olfactory neuroblastoma using the immunoperoxidase avidin-biotin complex method.

antibodies	source	dilution
Monoclonal:		
Cam 5.2	ICRF	1:5
LP 34	ICRF	1:5
synaptophysin	Dako, UK	1:20
chromogranin A	Dako, UK	1:50
MNF 116	Dako, UK	1:5
serotonin	Dako, UK	1:10
HMFG 2	ICRF	neat
HNK 1 (=CD67)	Beckton-Dickinson	neat
AUA 1	ICRF	neat
Polyclonal:		
protein gene protein (PGP) 9.5	Dako, UK	1:1,000
neuron-specific enolase (NSE)	Ultraclone, UK	1:4,000
S-100 protein	Dako, UK	1:1500
glial fibrillary acidic protein (GFAP)	Dako, UK	1:500
calcitonin	Eurodiagnostics	1:50
ACTH	Dako, UK	1:400

the first five cases in the series was by combined lateral rhinotomy and radiotherapy in four cases, and radiotherapy alone to one extensive lesion with cervical lymphadenopathy at presentation. All subsequent 15 cases underwent craniofacial resection (Cheesman et al., 1986). Of these, five had received radiotherapy pre-operatively, and one underwent a course of irradiation post-operatively. Thus, nine cases underwent craniofacial resection alone. Three patients subsequently underwent revision craniofacial surgery.

Immunohistochemistry was performed on paraffin-embedded material from 15 specimens taken from 10 patients. A panel of antibodies (Table 2) was applied to sections using the immunoperoxidase avidin-biotin complex method. Prior to incubation with the antibodies Cam 5.2, LP 34, MNF 116, AUA 1 and GFAP (glial fibrillary acidic protein) sections were trypsinized. Endogenous peroxidase activity was blocked with methanolic hydrogen peroxide. Appropriate positive and negative controls were used.

RESULTS

The series comprised 12 men and 8 women, their ages ranging from 14 to 67 years (mean 46 years; Table 1). Nasal symptoms of unilateral obstruction (88%), epistaxis (53%) and rhinorrhoea (41%) predominated, ocular (12%) and neurological (18%) problems were less common, and only one patient presented with cervical lymphadenopathy.

At surgery the tumour was found to involve the upper nasal cavity in all cases, but had extended to the ethmoid complex on one (or both) sides in 33%. Erosion of the cribriform plate or ethmoidal roof was macroscopically present in 60% (12/20) and could be shown histologically to infiltrate the olfactory bulbs in 60% of those undergoing craniofacial resection (9/15).

The results of the immunohistochemical staining are shown in Table 3. The general neuroendocrine marker neuron-specific enolase (NSE) stained all specimens, usually showing a strong, diffuse staining pattern (Figure 1). PGP 9.5 stained all but one specimen, with a similar pattern. Synaptophysin was less often positive, 11 specimens from 6 patients being positive. Chromogranin A stained 5 specimens and HNK 1 only 3, with less intense staining than the other neuroendocrine markers.

Two cases stained for cytokeratins using MNF 116 (which stains for cytokeratins 10, 17, and 18) and one with CAM 5.2 (which recognizes cytokeratins 8, 18, and 19), the staining with CAM 5.2 and in one case with MNF116 being a discrete perinuclear dot (Figure 2). This pattern of staining corresponds to bundles of perinuclear microfilaments seen with electron microscopy (Figure 3). No cases stained with LP 34, an antibody to high-molecular-weight cytokeratins. Calcitonin stained 2 tumours. ACTH and serotonin, the other neuropeptides tested, did not

Table 3. Immunohistochemical staining of olfactory neuroblastoma cases.

	patient no.														
antibody	5	6a	6b	6c	7	8a	8b	8c	9	10	11	12	13	14a	14b
Cam 5.2	–	–	+++	+	–	–	–	–	–	–	–	–	–	–	–
MNF 116	–	–	+++	–	–	–	+	–	–	–	–	–	–	–	–
LP 34	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
AUA 1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HMFG 2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NSE	++	+++	+++	+++	+	++	+++	+++	+++	+++	+++	+++	+++	++	+++
PGP 9.5	+	+++	+++	+++	+++	–	+++	+++	+++	+	+++	+++	+++	++	+++
synaptophysin	–	++	+++	+++	+	+	+++	+++	++	–	–	+	–	++	+
chromogranin A	–	–	–	–	–	–	+++	–	+	+	+	–	–	–	+
HNK 1	–	–	–	–	+	–	++	–	+	–	–	–	–	–	–
ACTH	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
calcitonin	–	–	++	+	–	–	+++	–	–	–	–	–	–	–	–
serotonin	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S-100 protein	–	–	+	–	+++	–	+++	–	+++	+++	+++	–	–	–	–
GFAP	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

+: weak staining; ++: moderate staining; +++: strong staining.

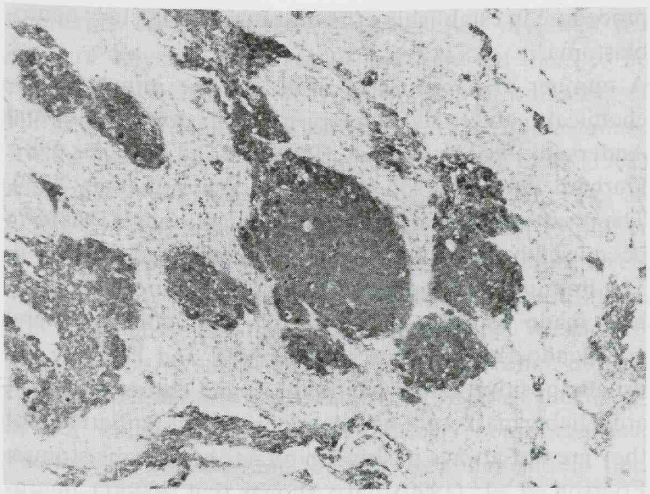

Figure 1. Light micrograph showing lobules of olfactory neuroblastoma staining strongly with neuron-specific enolase (immunoperoxidase avidin-biotin complex method).

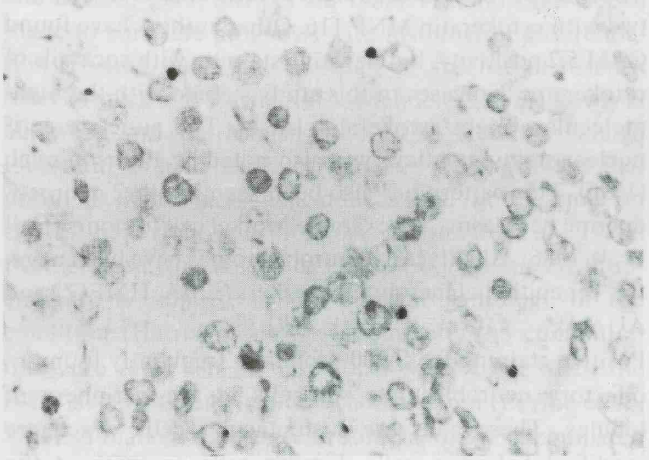

Figure 2. Light micrograph showing punctate perinuclear staining of olfactory neuroblastoma with Cam 5.2 (immunoperoxidase avidin-biotin complex method).

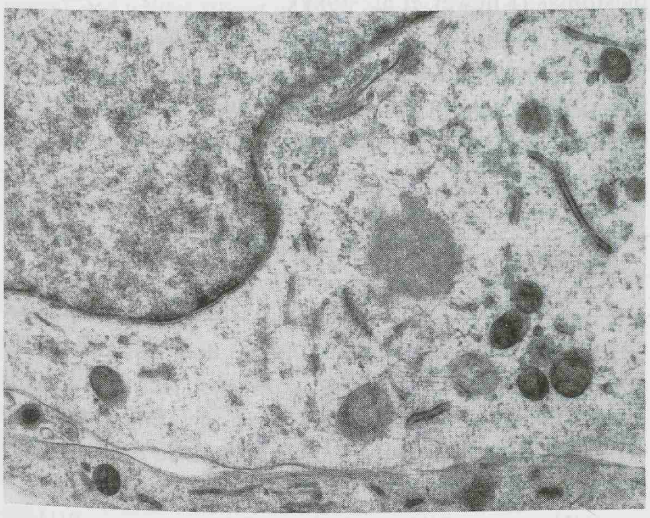

Figure 3. Electron micrograph of part of an olfactory neuroblastoma cell. In the cytoplasm is a bundle of microfilaments corresponding to the Cam 5.2 positivity seen by immunohistochemistry (×14,500; courtesy of Dr. Lucienne Papadaki).

stain any cases. The epithelial membrane markers HMFG 2 and AUA 1 were negative in every case. Antibodies to S-100 protein stained sustentacular cells in 6 cases. GFAP was negative in every case.

When the clinical outcome of the patients is considered, follow-up ranges from 10 months to 15 years with seven in excess of five years. Ten patients are alive and well (four with >5 years follow-up), two are alive with recurrent disease, five are dead (one of intercurrent disease), and three are lost to follow-up. When those fifteen patients undergoing craniofacial resection are considered, eight are alive and well, two alive with recurrent disease, four dead (one of intercurrent disease), and one lost to follow-up.

DISCUSSION

The age range and sex ratio of our patients is representative of the condition as judged by other published series (Kadish et al., 1976; Mills and Frierson, 1985; Schwaab et al., 1988), although some authors have found a greater number of younger patients (Bailey and Barton, 1975). The clinical presentation of these patients is also as expected, although the incidence of orbital symptoms was less in our group than in some other series (Levine et al., 1986; Schwaab et al., 1988). Diminished olfaction appears to be overlooked by patient and clinician alike, although is certainly present if specifically sought.

The site of the lesion is directly related to the distribution of the olfactory epithelium with local spread into adjacent areas, reflecting the surgical anatomy of the cribriform plate and superior lateral wall. Thus, the ethmoids are frequently involved, and the tumour can be assumed to have spread into the anterior cranial fossa along olfactory fibres even in the absence of bone destruction and macroscopical disease (Harrison, 1984).

The tumour is relatively rare with 250 cases being reported in the literature up to 1988 (Schwaab et al., 1988). However, olfactory neuroblastoma constitutes 4% of our series of 500 malignant tumours of the nose and paranasal sinuses, possibly reflecting our interest in craniofacial resection. The tumour's apparent rarity may be a consequence of under-diagnosis in the past when it may have been confused with other small-cell malignancies such as malignant melanoma, lymphoma, rhabdomyosarcoma, and poorly-differentiated squamous cell carcinoma.

Although changes in urinary vanillyl mandelic acid have been reported (Harrison, 1984), diagnosis has relied on histological criteria supplemented by electron microscopy. Olfactory neuroblastoma has a characteristic histological appearance, although diagnosis can be difficult with small specimens. The tumour often grows in a lobular pattern with congeries of blood vessels in close association to the tumour cells (Figure 4). The cells contain a small, round basophilic nucleus and scanty cytoplasm, and mitotic activity is usually low. Pseudo-rosettes may be seen and are a helpful diagnostic, but true rosettes are rare. Sometimes the tumour may be seen to arise from the olfactory epi-

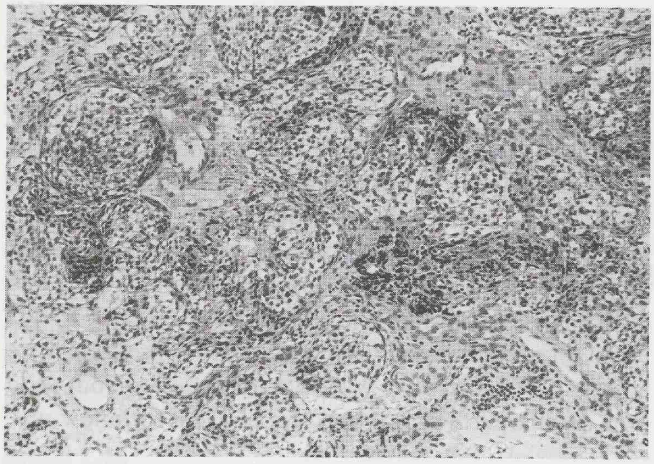

Figure 4. Light micrograph showing the characteristic lobular growth pattern of olfactory neuroblastoma (hematoxylin and eosin; ×100).

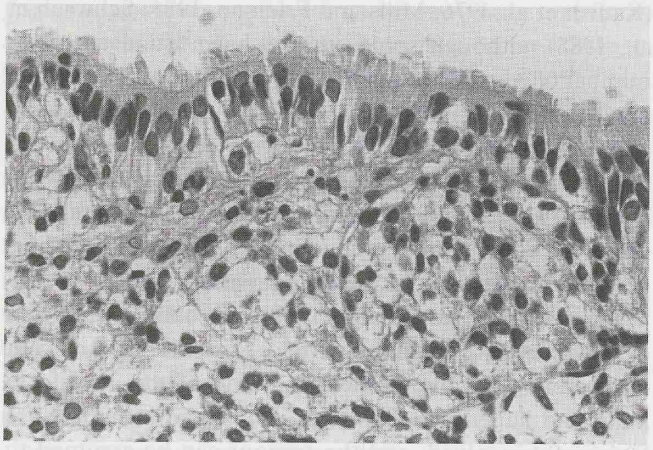

Figure 5. Light micrograph showing an olfactory neuroblastoma arising from olfactory neuroepithelium (hemotoxylin and eosin; ×400).

thelium (Figure 5). In less differentiated tumours the lobular architecture becomes less prominent or absent, and mitotic activity and cellular pleomorphism more pronounced. Diagnosis of these tumours has relied on the histological appearance supplemented with electron microscopy to demonstrate neuronal processes and neurosecretory granules. However, immunohistochemistry has now supplanted electron microscopy as the preferred procedure in establishing the diagnosis of olfactory neuroblastoma.

A number of papers have evaluated the immunohistochemical features of olfactory neuroblastoma (Choi and Anderson, 1985; Taxy et al., 1986; Axe and Kuhajda, 1987: Durham 1989; Frierson et al., 1990; Schmidt et al., 1990). The results of staining with the main antibodies that have been used by various authors are summarized in Table 4. The immunohistochemical diagnosis of neuroblastoma is best made using a panel of such antibodies. General neuroendocrine markers such as NSE and PGP 9.5 are usually positive, and synaptophysin and chromogranin A are reliable markers of neuroendocrine differentiation, but they are not always positive in olfactory neuroblastomas. Frierson et al. (1990) have shown that 200-kD neurofilaments and microtubules can be identified in most cases with appropriate antibodies. Olfactory neuroblastomas can be positive for cytokeratins, these proteins but are found in less than 50% of cases. In this study one case was positive with the low-molecular-weight cytokeratin CAM 5.2 and two with cytokeratin MNF 116. Other authors have found CAM 5.2 positivity, but negative staining with cocktails of cytokeratin. No cases in this study stained with the high-molecular-weight cytokeratin LP 34. The punctate perinuclear staining pattern was also noted by Frierson et al. (1990). This pattern has also been seen in other neuroendocrine neoplasms, for example Merkel cell tumours (Hall et al., 1986). All olfactory neuroblastomas have been negative for epithelial membrane markers (EMA, HMFG 2 and AUA 1).

Positive staining for S-100 protein is frequently found in olfactory neuroblastoma, in cells at the periphery of lobules. These cells are sustentacular cells which are modified Schwann cells. Similar cells are seen at the periphery of the lobules ("Zellballen") of paragangliomas and phaeochromocytomas. In paragangliomas these cells also stain with GFAP, but do not do so in olfactory neuroblastomas (Kliewer et al., 1989).

In 1980, Kameya and colleagues described 4 cases of neuroendocrine carcinoma of the paranasal sinuses, and in 1982 Silva et al. described a series of 20 tumours of the upper nasal cavity which were classsified as neuro-

Table 4. Immunohistochemistry findings from published series.

	antibodies								
authors	NSE	synaptophysin	chromogranin A	Leu-7	cytokeratin	S-100	GFAP	neurofilament	EMA
Choi and Anderson (1985)	10/10	–	–	–	1/10	10/10	–	–	–
Taxy et al. (1986)	20/27	–	–	–	10/25	15/27	–	4/27	–
Axe and Kuhajda (1987)	6/8	–	1/8	–	0/8	5/8	0/8	1/8	0/8
Wick et al. (1988)	–	5/5	–	–	0/5	0/5	–	–	0/5
Durham (1989)	10/14	–	6/10	8/10	4/10	10/14	–	–	–
Frierson et al. (1990)	11/11	7/11	1/11	–	4/11	8/11	1/11	7/11	0/11
Schmidt et al. (1991)	4/4	4/4	1/4	–	0/4	4/4	0/4	0/4	–
this study	10/10	6/10	5/10	3/10	2/10	6/10	0/10	–	0/10*

*: HMFG 2 and AUA 1.

endocrine carcinoma. Furthermore, they reviewed the cases of Kayema et al. (1980) and reported them to be identical to their cases. It is the opinion of a number of authors that these tumours are olfactory neuroblastomas. It is of interest that two of the cases of Silva et al. (1982) stained for ACTH and one of the cases of Kameya et al. (1980) had high serum levels of ACTH and MSH. Another of the cases of Kameya et al. (1980) had high serum levels of calcitonin, calcitonin was extracted from the tumour, and it was demonstrated by immunostaining. We did not see any positive staining for ACTH or serotonin, but did find positive staining for calcitonin in two cases, the histology of which were typical of olfactory neuroblastoma. Positive immunostaining for neuropeptides should not, therefore, exclude a diagnosis of olfactory neuroblastoma. The natural history of the condition is characterized by local recurrence and secondary spread to cervical lymph nodes, brain, spine and lungs, although the incidence of metastasis has been variously estimated at 20–62% (Bailey and Barton, 1975; Silva et al., 1982). Attempts have been made to correlate prognosis with histological differentiation (Hyams, 1982), the presence or absence of rosettes (McCormack and Harris, 1955; Mendeloff, 1957; Lewis et al., 1965), and site (Kadish et al., 1976). However, a large long-term French study of 45 cases demonstrated that different histological appearances had no bearing on clinical behaviour and prognosis (Schwaab et al., 1988). Furthermore, the ability of the tumour to spread intracranially is implicit in the surgical pathology of the condition (Harrison, 1984) and supports the contention that cure rates can be dramatically improved when the issue is addressed by craniofacial resection (Levine et al., 1986). A marked difference in survival of 37.5% compared to 82% was found when Levine et al. (1986) divided their patients into those treated without and those with craniofacial resection respectively. However, the follow-up was much shorter in our craniofacial group (with a mean of four years) and whilst 10 out of 14 of our patients are known to be alive following craniofacial resection, long-term follow-up is essential when death can occur even after 10 years from treatment. Thus whilst 71% (5/7) of our patients with >5 year follow-up are alive, the other two patients succumbed at 10 and 8.5 years, respectively. This, and the small numbers of patients at present, compromises any definitive evaluation of prognosis.

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