Decisive role of immunocytochemistry in aspiration cytology of chordoma of the clivus: A case report with review of the literature

Giorgio Gherardi, M.D., M.I.A.C.*, Cristina Marveggio, Sc.D.*, Claudio Cola, M.D.†, Gianantonio Redaelli, M.D.†

Abstract

Immunocytochemistry (ICC) proved to be an essential adjunct in the fine-needle aspiration (FNA) cytological diagnosis of chordoma of the clivus in a 62-year-old woman. The cytological picture in routinely stained smears was not entirely diagnostic for chordoma due to the paucity of typical 'physalipherous' cells. To exclude other primary or metastatic neoplasms of the skull base possibly sharing the same cytological picture, additional direct smears were immunostained with antibodies specific for cytokeratin (CK), vimentin (VIM), S100 protein (S100P), carcinoembrionic antigen (CEA), epithelial membrane antigen (EMA), glial fibrillary acidic protein (GFAP), CD68 antigen (KP1) and with the 'panepithelial' antibodies B72.3 and Ber-EP4. Chordoma cells showed the following immunoprofile: $CK^+/VIM^+/S100P^+/CEA^-/EMA^+/GFAP^-/B72.3^-/Ber-EP4^-/CD68^+$. The pattern of immunoreactivity for CK, S100P and CEA confirms previously reported data, while the $B72.3^-/Ber-EP4^-/CD68^+$ staining profile represents a novel observation. The detection of a $CK^+/S100^+/CEA^-/B72.3^-/Ber-EP4^-$ immunocytological profile of chordoma cells in aspirates is a basic requirement to exclude pertinent diagnostic differentials, such as metastatic carcinoma, ependymoma and sarcoma, and permits a reliable pre-operative diagnosis of the tumour by aspiration cytology.

Key words: Immunohistochemistry; Biopsy, needle; Head and neck neoplasms; Chordoma

Introduction

Chordoma is a rare bone neoplasm of the head and neck area, where it occurs mainly in the clivus, sphenoid area, upper and lower nasopharynx (Heffelfinger *et al.*, 1973; Perzin and Pushparaj, 1986). The conventional protocol for evaluation of this tumour involves open biopsy for tissue acquisition and histopathological investigation, however the frequent location of the lesion at the base of the skull can preclude an easy access for open surgical biopsy. FNA biopsy represents a reliable alternative modality for achieving expedient, minimal intervention, conservative cost, tissue-equivalent diagnostic information and it is possible to trace 16 previous reports describing the cytopathological diagnosis of 48 cases (Carvalho and Coelho, 1974; Elliott *et al.*, 1983; O'Dowd and Schumann, 1983; Xiaojing and Xiangcheng, 1985; Finley *et al.*, 1986; Kontozoglou *et al.*, 1986; Rone *et al.*, 1986; Apaja-Sarkkinen *et al.*, 1987; Layfield *et al.*, 1987; Nijawan *et al.*, 1989; Plaza *et al.*, 1989; Angelpulos *et al.*, 1990; Perasole *et al.*, 1991; Walaas and Kindblom, 1991; Hughes *et al.*, 1992), 12 of which were located in the head and neck (Carvalho and Coelho, 1974; Finley *et al.*, 1986; Apaja-Sarkkinen *et al.*, 1987; Walaas and Kindblom 1991). Moreover, the peculiar immunocytochemical profile of chordoma cells, which has been also evaluated in cytological preparations of 13 previously reported cases (Finley *et al.*, 1986; Kontozoglou *et al.*, 1986; Plaza *et al.*, 1989; Walaas and Kindblom, 1991; Plate and Bittinger, 1992) facilitates differentiation of the tumour from other primary or metastatic neoplasms occurring in bone.

We report a case of chordoma of the clivus initially diagnosed by FNA cytology and ICC. Our findings confirm the previously published immunocytochemical properties of chordoma cells and provide additional data which demonstrate the reliance of ICC coupled to cytomorphological observations in the pre-operative diagnosis of the tumour.

Case report

A 62-year-old woman presented with nasal obstruction, and a history of recurrent nasal discharge and epistaxis, snoring and dysphagia of one-year duration. A soft and friable tumour mass was detected in the nasopharynx by indirect nasopharyngoscopy. Magnetic resonance imaging (Figure 1) demonstrated massive erosion with destruction of the clivus, erosion of the anterior arch of the atlas bone and of the odontoid process of the axis bone, due to an expansive tumour extending downward as a soft tissue mass in the nasopharynx. The tumour caused a slight reduction of the subarachnoid spaces around the pons and bulbus, but no compression of these structures. FNA biopsy coupled to immunocytochemical findings was diagnostic for chordoma. The patient underwent an infratemporal subtotal excision of the mass with subsequent high-dose radiotherapy.

Histopathological evaluation of the surgical specimen allowed confirmation of the diagnosis of chordoma. She is alive and well 10 months after intervention.

Material and methods

The percutaneous FNA biopsy was performed using a 23-gauge CHIBA biopsy needle (Sterylab s.p.a., Milano, Italy) attached to a 20 cc syringe, under direct visualization of the pharyngeal portion of the mass through the oral cavity. No anaesthesia was required. The aspirated material was immediately

FIG. 1

Magnetic resonance imaging (MRI) demonstrating an osteolytic, midline, destructive and expansive tumour mass mainly located in the clivus but also extending into the nasopharynx.

FIG. 3

A multinucleated cell with 'bubbly' vacuolated cytoplasm, having a physaliperous-like appearance, is seen along with mononucleated cells with varying degrees of cytoplasmic vacuolization. (R stain; ×330).

smeared onto clean glass slides and four direct smears prepared: two were immediately immersed in 95 per cent ethanol and two were air-dried. Immediate evaluation of two slides stained, respectively, with quick Papanicolaou (P) and Romanowsky (R) stains helped anticipate the need for immunostaining. Two additional passes were performed thereafter, and eventually 12 ethanol-fixed direct smears became available for immunostaining. The procedure of immunostaining and of control validation has been described elsewhere (Gherardi and Marveggio, 1992). Smears were examined using the avidin–biotin–complex (ABC) for the demonstration of CK 'cocktail' (CK22, prediluted monoclonal: Biomeda Corp., Foster City, CA, USA), EMA (prediluted monoclonal: Biomeda), CEA (prediluted monoclonal: Biomeda), VIM (prediluted monoclonal: Dakopatts, Copenhagen, Denmark), S100P (prediluted polyclonal: Dakopatts), GFAP (prediluted monoclonal: Biomeda), epithelial antigen, Ber-EP4, (1:30 dilution, monoclonal: Dakopatts), human tumour-associated glycoprotein 72 (B72.3 prediluted monoclonal: Signet Laboratories, Inc., Dedham, MA, USA), KP1/CD68 (1:100 dilution, monoclonal, Dakopatts). The ABC method was performed using the Autoprobe III kit (Biomeda) with overnight incubation at 4°C of the primary antibodies. The same panel of antibodies was tested on tissue specimens from surgical intervention fixed in 10 per cent (v/v) buffered formalin, as routine, and embedded in paraffin. For purposes of compari-

son the immunoreactivity of B72.3, Ber-EP4 and KP1/CD68 was tested also on histological sections of paraffin-embedded tissue specimens of two archival cases of chordoma of the sacrococcygeal region fixed with buffered formalin. KP1/CD68 immunoreactivity was also tested on a touch preparation smear of one of these latter cases. Immunostaining of this slide was performed after removal of the coverslip and rehydratation.

Results

Cytological findings

Smears showed a moderate cellularity. The background contained red cells, polymorphonuclear leucocytes and an abundant mucoid matrix which stained light green in P-stained smears or strongly purple in R-stained preparations. In these latter, the matrix was clearly seen, and appeared homogeneous, finely granular, or fibrillar (Figure 2). Tumour cells were seen in small sheets, or as single, noncohesive elements, and were interspersed with fibrils of mucoid extracellular matrix; cellular overlap was infrequent (Figure 2). Three types of tumour cells could be recognized: (1) a large cell with a round or oval configuration, that contained two or more nuclei and abundant cytoplasm or with a wispy or vacuolated cytoplasm (Figure 3); (2) a medium-sized and mononucleate cell having an epithelioid appearance and with similar cytoplasmic features (Figures 2, 3 and 4); (3) a mononucleate cell with a spindly configuration and nonvacu-
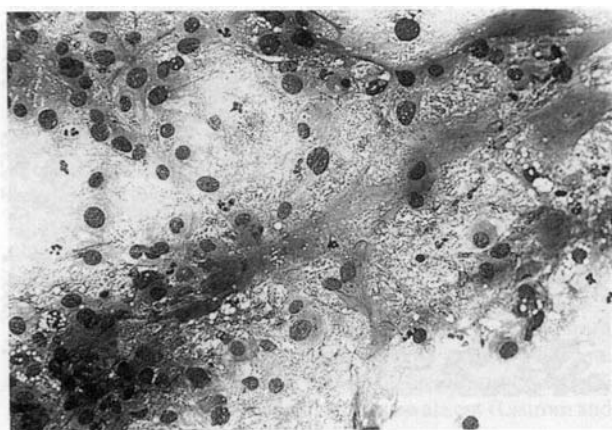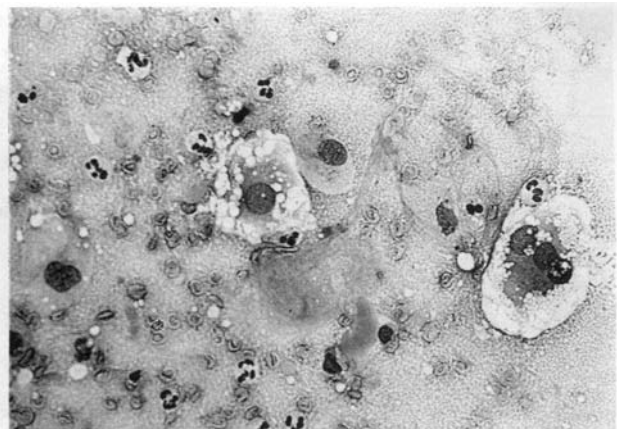

FIG. 2

The fine-needle aspirate consisting of medium-sized epithelioid cells, with round nuclei and varying degrees of cytoplasmic vacuolar change. Cells are loosely clustered or isolated, and are embedded in an abundant mucoid ground substance which appears to be made of condensed strands or a network of basophilic fibrils. (R stain; ×155).
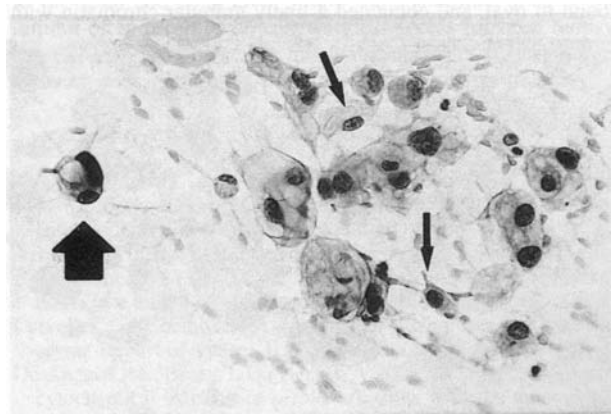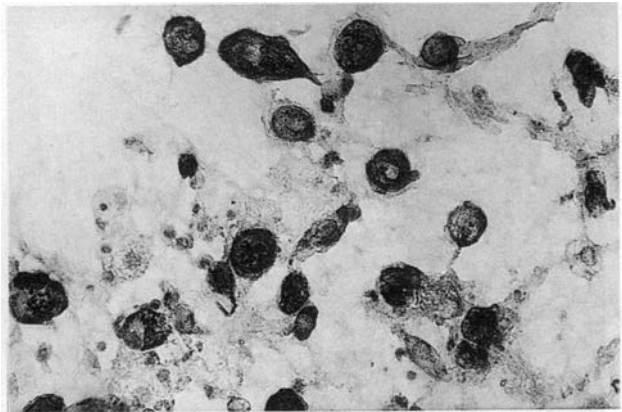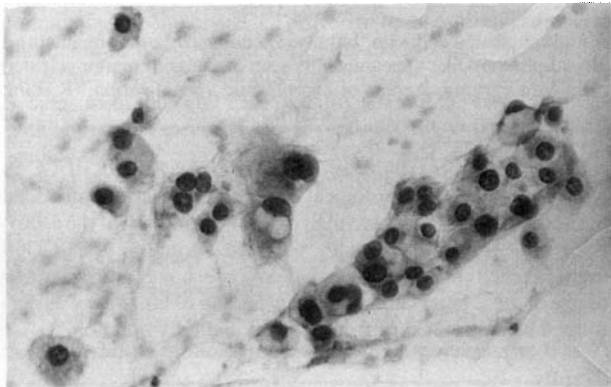

FIG. 4

Several tumour cells showing conspicuous cytoplasmic non-vascuolization. A signet-ring simulating cellular configuration is sometimes observed (large arrow). Two cells (small arrows) have a spindle shape. (P stain; ×150).

(a)

(b)

Fig. 5

(a) All cell types show strong cytoplasmic immunoreaction for anti-CK antibody. (ABC, counterstained with haematoxylin; ×170). (b) A negative immunoreaction for B72.3 antibody is seen in all cells. (ABC, counterstained with haematoxylin; ×170).

olated cytoplasm (Figure 4). The medium-sized epithelioid cells were highly prevalent and cells with a definite 'physalipherous' appearance were sparse. Cytoplasmic vacuoles appeared empty both on P and R stains, varied in size, and predominated in the peripheral portion of the cell types described here; hugh vacuolar transformation with a 'bubbly' cell appearance was not unusual (Figure 3). Sometimes cytoplasmic vacuolization was responsible for a signet ring configuration (Figure 4). Cytoplasmic borders were distinct and sharp in all cell types. Nuclei were always round to oval, and contained a finely granular chromatin with
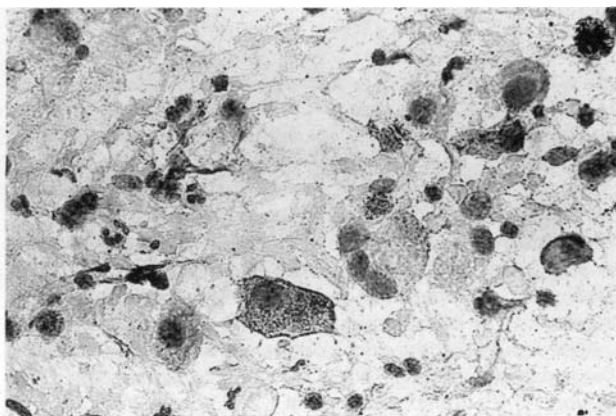

Fig. 6

Immunopositivity for KP1/M6/CD68 antibody is seen in a physalipherous-like cell which appears to be made of multiple granular deposits. (ABC, counterstained with haematoxylin; ×230).

multiple chromocentres. Pronounced nucleoli were rarely seen while pseudonucleoli, i.e. intranuclear cytoplasmic invaginations, were not infrequent.

Immunocytochemistry

Cytoplasm of tumour cells, in smears, stained positive with antibodies directed to CK (Figure 5a), EMA, S100P, VIM while it showed no reaction with anti-CEA, anti-GFAP, Ber-EP4 and B72.3 (Figure 5b) reagents. Immunoreaction for CK, VIM and EMA was seen in almost all the cells and sometimes prevailed along the plasma membrane. S100P immunopositivity was less intense and focal. KP1/CD68 immunopositivity was seen in the large majority of cells and appeared characteristically granular (Figure 6). Chordoma cells in an archival touch preparation showed the same pattern of immunoreaction for KP1/CD68 reagent.

Histopathology and immunohistochemistry

The surgical specimen consisted of multiple soft, gelatinous, grey-white tissue fragments, the largest measuring $2.5 \times 1.2 \times 1.2$ cm. Microscopically, the tumour was divided into lobules composed of clear cells growing in an abundant mucoid ground substance. Cellular morphology correlated well with aspiration cytology findings, while typical 'physalipherous' cells were more evident in tissue sections. Tumour cells grew as cords, clusters or pseudoacini, and lobules were separ-

(a)
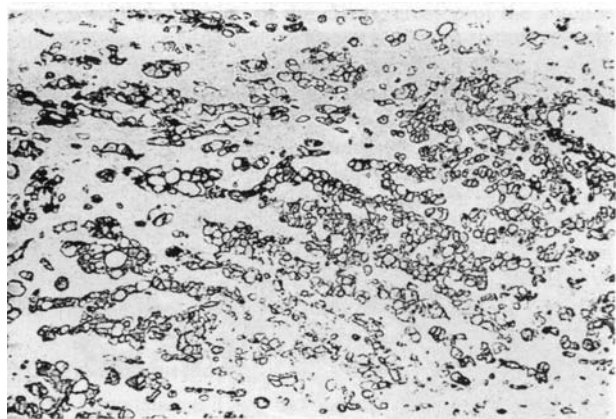
(b)

Fig. 7

(a) Histology demonstrating the tumour is composed of clear cells, growing in cords, and trabeculae embedded in a mucoid background. (H&E; ×32). (b) Immunohistochemical investigation showing strong cytoplasmic expression of CK in tissue section. (Not counterstained; ×35).

TABLE I
RESULTS OF THE IMMUNOCYTOCHEMICAL ANALYSIS ON NINE CASES REPORTED IN THE LITERATURE

Reference	No. of cases	Immunocytochemical results										
		CK	AE1	AE3	Cam 5.2	CEA	NSE	S100P	EMA	VIM	GFAP	NF
Finley et al., 1986	1		+	±		−	/	±	+	+	−	/
Plaza et al., 1989	1	+				−	/	+	/	/	/	/
Walaas and Kindblom, 1991	4				+	−	/	±	+	+	/	/
Perasole et al., 1991	1		+	+		−	+	−	+	/	−	/
Plate and Bittinger, 1992	2	+				/	/	+	/	+	−	+

CK: anticytokeratin antibody with wide spectrum specificit; AE1, AE3, Cam 5.2: anti-AE1, anti-AE3, Cam 5.2 anticytokeratin antibodies; CEA: anti-carcinoembryonic antigen antibody; NSE: anti-neuron-specific enolase antibody; S100P: anti-S100 protein antibody; EMA: anti-epithelial membrane antigen antibody; VIM: anti-vimentin antibody; GFAP: anti-glial fibrillary acidic protein; NF: anti-neurofilaments antibody. +: positive immunoreaction; ±: focally immunoreactive; −: negative immunoreaction; /: not investigated.

ated by fibrous bands (Figure 7a). No areas of chondroid transformation were observed. Immunohistology confirmed the immunocytological findings: most of the cells were immunoreactive for CK (Figure 7b), VIM, EMA, some were S100P-positive, and none were immunopositive for CEA, GFAP, B72.3 and Ber-EP4. Positive immunoreaction for KP1/CD68 was confirmed in tissue sections of the current case and of two additional archival cases of chordoma.

Discussion

The two most important cytological features of chordoma in aspirates are the presence of vacuolated cells and background myxoid stroma; typical 'physalipherous' cells are very helpful for diagnosis but are not constantly present (Carvalho and Coelho, 1974; Elliott et al., 1983; Finley et al., 1986; Kontozoglou et al., 1986; Rone et al., 1986; Walaas and Kindblom, 1991). Our findings in the case under discussion recapitulated most of the typical cytomorphological features of chordoma, but due to paucity of typical 'physalipherous' cells the overall picture was originally considered not specific enough to exclude other diagnostic differentials.

In the head and neck region the differential diagnosis of chordoma based on the cytological picture described in this paper should mainly encompass chondroma or 'low grade' chondrosarcoma, myxoid liposarcoma, myxopapillary ependymoma and clear cell-mucinous carcinomas (Finley et al., 1986; Rone et al., 1986; Apaja-Sarkkinen et al., 1987; Plaza et al., 1989; Walaas and Kindblom, 1991; Hughes et al., 1992; Plate and Bittinger, 1992). The application of ICC to fine-needle aspiration cytological specimens can help in the differential diagnosis and offers the potential for making a specific diagnosis.

A review of the literature demonstrates that in nine aspirates studied pre-operatively by ICC (see Table I) chordoma cells were characterized by positive immunostaining for CK, S100P, EMA and VIM, and negative immunoreaction for CEA. Kontozoglou et al. (1986) studied the immunocytochemical profile of chordoma cells aspirated from four additional excised surgical specimens and obtained similar results. According to Plate and Bittinger (1992) and Walaas and Kindblom (1991) this immunoprofile can reliably exclude the soft tissue tumours (described in this paper) and ependymoma mainly because those entities do not express CK, but we would argue that it does not provide any substantial evidence with which to differentiate chordoma from metastatic carcinoma. In fact, in carcinoma cells, S100P and VIM are rarely expressed, CK and EMA are almost constantly present, and CEA immunopositivity can be absent (Listrom and Fenoglio-Preiser, 1992).

To differentiate chordoma from metastatic carcinoma a more extensive immunocytochemical investigation is required, if possible. We had the opportunity to test the additional reactivity of chordoma cells with the antibodies B72.3 and Ber-EP4. Immunopositivity for B72.3 and Ber-EP4 is seen in most types of adenocarcinoma cells and these reagents have been widely

used as 'panepithelial' markers in the cytological typing of malignant cells in serous effusions (Nance and Silverman, 1991; De Angelis et al., 1992; Listrom and Fenoglio-Preiser, 1992). In the case under study, chordoma cells showed a negative immunoreaction for B72.3 and Ber-EP4 both on smears and on tissue sections. The lack of immunoreactivity for these reagents in tissue sections of two additional archival cases confirms the above observation and validates the hypothesis that chordoma cells do not express the 'panepithelia' markers. Thus, it is possible to conclude that in aspirates the $CK^+/S100P^+$ immunoprofile, coupled to a negative immunoreaction for CEA, B72.3 and Ber-EP4, can effectively exclude metastatic carcinoma in the differential diagnosis of chordoma.

In this study we could also test the immunopositivity of chordoma cells for the antibody KP1 which recognizes the CD68 antigen (Micklem et al., 1989), a panmacrophage marker, in paraffin sections (Pulford et al., 1989). Cells showed a cytoplasmic immunopositivity for KP1/CD68 in both cytological and histological preparations of the case under study, as well as on histological and cytological specimens of two additional archival cases of chordoma. The immunoreaction was typically granular. This is, to our knowledge, a hitherto undescribed observation which may be in keeping with the additional reactivity of this antibody with some non-macrophage-derived tumours in cytological specimens (Doussis et al., 1993).

Conclusions

Our findings expand the contribution and role of FNA in the diagnosis of chordoma. A correct pre-operative diagnosis can be accomplished by aspiration biopsy, and the application of ICC to direct smears plays a decisive role in the differential diagnosis. In the head and neck area, where the anatomical sites of the tumour often preclude an easy surgical access for open biopsy, the needle aspirate offers the invaluable option of an initial diagnosis on which therapeutic strategies can be planned.

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Address for correspondence:
Dr Giorgio Gherardi,
Istituto di Anatomia e Istologia Patologica,
Ospedale Civile,
25, Via Stelvio,
23100 Sondrio,
Italy.