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Annals of Clinical Cytology and Pathology

Research Article

Chordomas; Crush Intraoperative Analysis

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Submitted: 22 February 2015

Accepted: 12 April 2015

Published: 13 April 2015

Keywords

- Chordomas
- Crush intraoperative study
- Smears
- Brain tumors
- Cytomorphologic

Abstract

Chordomas are known as rare primary malign tumours that have formed from primitive notochord remains and exhibit different epithelial properties. Morphologically they have distinct cell borders; the nucleus displays a monotony and blandness. Pleomorphsm, atypia, anaplasia and hipercromasia were minimal. Vacuoles were seen in cytoplasm. During January 1995 until June 2005, 22 surgeries of chordomas and intraoperative crush were performed. The background was dirty with myxoid appearance, myxoid matrix growing in sheets or cord and had vacuoles showed more evidence of physaliferous cells. Chondoid chordoma showed vacuolated cells as well as other cells with eosinophilic cytoplasm and myxoid background. Singles cells were observed only in two cases and high cellularity was observed in 8 (42%) cases. Two cases were frank errors, only one had a partial correlation. The percentage of mistake was 30% and our accuracy diagnosis was 70%. The correlation with clinical details and radiological findings were helpful in improving the accuracy rate. There was no differentiation between cytomorphologic features smear of intracranial tumors vs sacrococcygeal location. Intraoperative analysis of chordomas could be helpful to have a good diagnosis and to have a better surgical resection.

INTRODUCTION

Chordomas are type of tumours which originate from the remnants of the notochord. Originally chordomas represent 1-4% of all primary malignant bone tumors [1]. They constituted the 5% of all neoplasms and 86% of bone tumors, [2] about one-half of chordomas are located in the sacro-coccygeal region and approximately 30%-35% are present at the base of the skull [1]. However can occur anywhere along the vertebral column. Chordomas are rarely seen in children or adolescents. Men are affected more frequently than women [1]. Originally chordoma it is considered as an intermediate grade malignant bone tumour [3] and have low tendency to metastasis and have a poor prognosis in long-term follow-up. Metastasis is seen in 5–40% of chordoma cases [2,3].

There are three histological variants of chordoma: classical (or conventional) chondroid and dedifferentiated [1,2]. The cells have small round nuclei and abundant vacuolated cytoplasm, sometimes described as physaliferous (having bubbles or vacuoles) [1,2].

Macroscopically, those tumors are lobulated gelatinous and brownish-grey in colour, and occasionally appear translucent [1-4]. Histologically, these tumors are characterized by the presence of physaliphorious cells which are very rich in mucin and glycogen [1-3]. Tissue section of the aspirate sample shows sheets of vacuolated "physaliphorious" cells, classic of chordoma [2-4].

Intraoperative smear cytology provides a rapid and reliable intraoperative diagnosis and guidance to the neurosurgeon during surgical resection and lesion targeting. It also helps the surgeon to monitor and modify the approach at surgery. Smear cytology is of great value in intraoperative consultation of central nervous system tumors [5]. The smear technique is challenging for a neuropathologists where rapid and accurate diagnosis is to be given on small biopsies and conducted to assess the usefulness, accuracy and the diagnostic pitfalls of smear diagnosis [5]. Squash smear technique is a very reliable and rapid method of intraoperative diagnosis. Knowledge of clinical and neuroimaging details helps the experienced neuropathologists to improve the diagnostic accuracy [5]. The ideal intraoperative method used, should be accurate, rapid and should allow preservation of tissue for paraffin embedded sections. The frozen section technique is less popular for brain biopsies as it uses a substantial amount of tissue and produces freezing artefacts [5,6].

We retrospectively reviewed 22 cases from patients diagnosed by crush intraoperative smear of chordoma focusing

on the cytomorphologic features of the tumors. Cytological and histological correlation.

MATERIAL AND METHODS

Clinical cases

A search of the archival database over a 10-year period (1995-2005) at the Department of Neuropathology at National Institute of Neurology and Neurosurgery, Mexico city. 22 cases of chordomas included in this study. All the tumors were intraoperatory analysed. Samples were obtained by direct and visible masses or under Computed Tomographic (CT) imaging guidance in the deep-seated brain neoplasms Cytological studies included evaluation of aspirated material from the primary tumor and correlation ship with the definitive biopsy diagnosed.

A histologic and cytologic correlative retrospective review was performed to assess the ability of cytology to render an accurate and specific diagnosis of this malignancy. All samples were obtained by direct and visible masses or under Computed Tomographic (CT) imaging guidance in the deep-seated brain neoplasms (Figure 1a).

For all specimens, slides were air dried immediately for hematoxilin and eosin stain. Smear preparation, and preliminary microscopic interpretation were performed immediately in all cases. A cell block preparation was obtained for most of the cases that were embedded in paraffin. There was histological confirmation in all cases.

The cases were evaluated for the following cytomorphological parameters: cellularity (scanty, moderate, high, cellular arrangement or in single cells), sheets or clusters formation, prominent nucleoli (present or absent), nuclear molding (present or absent), nuclear-to-cytoplasmic ratio (low, moderate, high), and background material (inflammatory, necrotic, bloody, mucinous, or clean). Discrepancies in interpretation were resolved by forum of discussion. Overall, interobserver agreement was high. Cytological features and causes for discrepancy of the final diagnosis were reviewed and noted. At the time of review, all cases had tissue correlation with the surgical resection specimen of the primary tumor.

RESULTS

Twenty-two cases were included in this study, with clinical and radiological diagnosis of chordomas who underwent biopsy and who underwent intraoperative study crushed. The specific tumor subtype on cytology, in cases in which an attempt was made to further refine the diagnosis, was erroneous in four cases. The only false-negative diagnosis was in 3 cases and resulted from rendering a definite diagnosis on insufficient material. The cytological findings are summarised in Table 1.

19 cases corresponded to classic type and three was chordoid type. Sixteen cases (73%) were male and 6 (27%) female, aged from 15 to 86 years (median 49 year). Tumor localization: 12 (55%) cases were located intracranial and 10 (45%) in sacrococcygeal location.

Neoplastic cells are very fragile; they usually disintegrate and break at the time of preparation (Figure 2a and 2b). The cell exhibit epithelial properties, have distinct cell borders (Figure 2b), abundant cytoplasm and are adherent to each other (Figure 2c), can see in isolated cell (Figure 2d) or in sheets or in slightly eosinophilic cord (Figure 2d). The nucleus displays a monotony and blandness (Figure 2e). Pleomorphsm, atypia, anaplasia and hypercromasia were minimal. Vacuoles were seen in cytoplasm (Figure 2e). The background was usually dirty with myxoid appearance, myxoid matrix growing in sheets or cord and had vacuoles (Figure 2f). Chondoid chordoma always showed myxoid

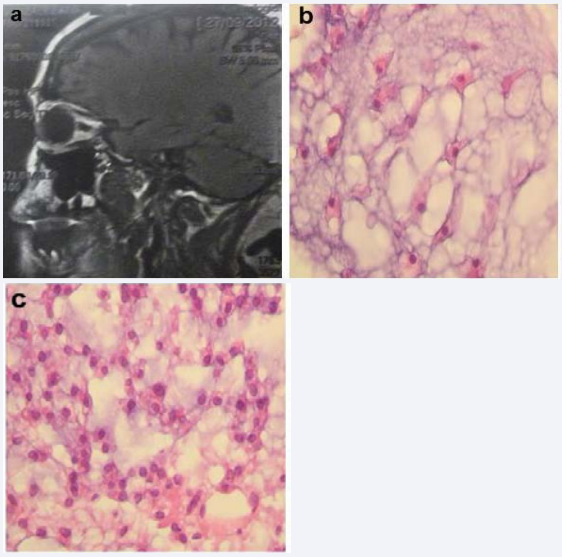

Figure 1 (a) Showed a MRI in sagittal imaging that showed enhancement tumour in skull base and in (b) Histologically, these tumors is characterized by the presence of physaliphorious cells which are very rich in mucin and glycogen, in (c) observed a chordoid chordoma features (H&Ex400).

Table 1: Cytomorphological characteristic of chordoma smears.

	Classic Chordoma n=19(%)	Chondoid Chordoma n=3(%)
Cellularity		
Scanty,	1(59)	1(33)
Moderate,	10(53)	1(33)
High	8(42)	1(33)
Arrangement		
Single cells,	1(5)	0
Sheets	8(42)	1(33)
Clusters	10(53)	2(67)
Cellular prominent nucleoli		
Present	8(42)	2(67)
Absent	11(58)	1(33)
Nuclear molding		
Present	12(63)	1(33)
Absent	7(37)	2(67)
Atypia	8(42)	3 (100)
Pleomorphism	5(26)	3(100)
Background material		
Fibrillary	1(5)	1(33)
Granular	19(100)	3(100)
Haemolytic	3(16)	1(33)
Myxoid	19(100)	3(300)
bloody	3(16)	3(100)
Inflammation	5(26)	1(33)

background (Figure 3a and 3b), some cells showed eosinophilic vacuoles (Figure 3c), as well as other cells with eosinophilic cytoplasm (Figure 3d) and perinuclear halos are seen in some cells (Figure 3d). Cytoplasm is usually thin, fine granulated and radiated (Figure 3e), also nuclear atypism with an isonucleosis and seen (Figure 3f). Generalities of smear were observed in Table I. Singles cells were observed just in two cases (9%), high cellularity was observed in 8 (42%) and moderated cellularity was in 12 (54.5%). The error percentage was 30%. Correlation with clinical details and radiological findings were helpful in improving the accuracy rate. With an accuracy of diagnosis correlation from 70%. Overall, inter observer agreement was high. There was no differentiation between cytomorphologic smear of intracranial tumors vs sacrococcygeal location, neither classical *vs* chondroid subtype. However Chondoid type showed more eosinophilic cells than classic type.

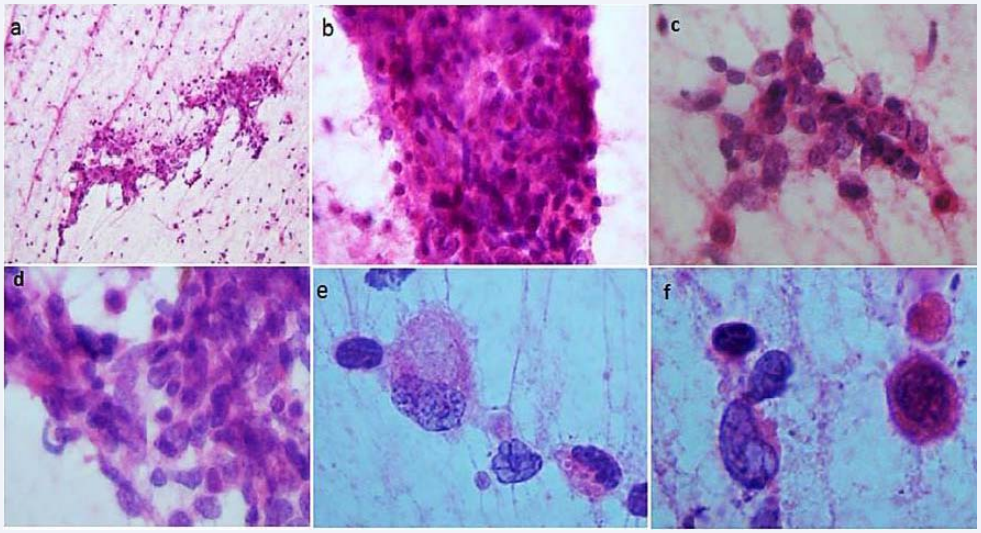

Figure 2 (a) Chordoma smears at low power observed a few cells in a myxoid background (H&Ex200). (b) Moderate cellularity observed with predefined of mixed background (H&Ex200). (c) Singles cells demonstrates the seemingly syncytial appearance of this tumour cells (H&Ex400). And in (d), observed a high cellularity, cell with epithelial appearance with abundant clear cytoplasm. The smear produces thick cytoplasmic bridges among small cell group (H&E x400). (e) Smear showed single cells with abundant clear and vacuolated cytoplasm and homogeneous nuclei (H&Ex400). (f) Close up observed two cell types of benign appearance (H&Ex1000).

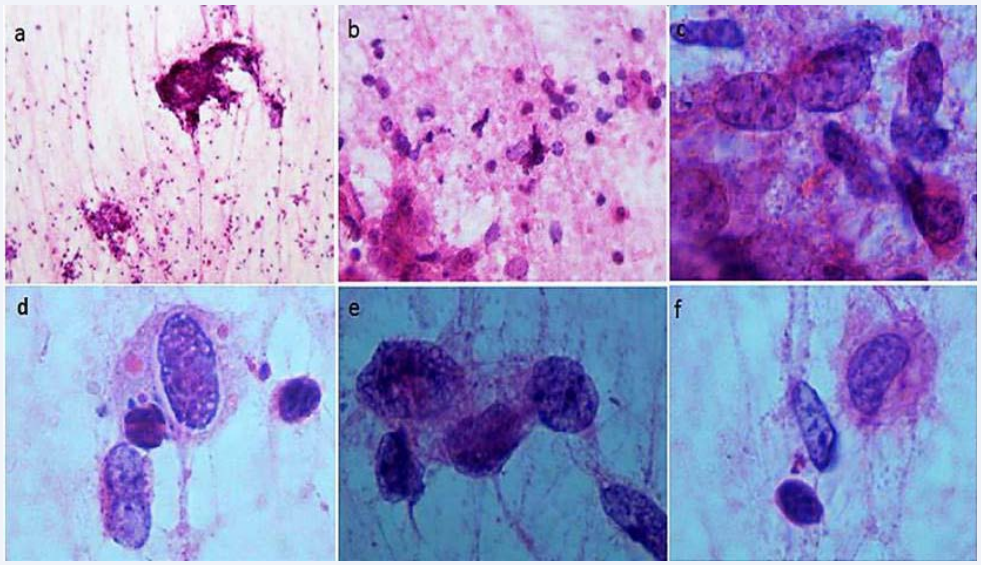

Figure 3 Chondroid Chordoma at low power smears showed tumour cell that were arranged in sheets, clusters with chondroid stroma. (b) Neoplastic cells that are very fragile and disintegrate background and break at the time of preparation. The background is dirty with myxoid appearance, myxoid matrix (H&E x200). (c) The cell exhibit epithelial proprieties, have distinct cell borders, abundant cytoplasm and are adherent to each other with granular appearance, in (d) observed isolated cells with eosinophilic cytoplasm and clear appearance of halos perinuclear and vascular cytoplasm (H&Ex400). (e) Observed some isolated cells that cytoplasm is usually thin, fine granulated and radiated, and in (f) also nuclear atypism with anisonucleosis and seen (H&Ex1000).

DISCUSSION

Eisenhardt and Cushing were the first to describe the cytological diagnoses in brain tumors in 1930 of touch preparations stained with supravital dyes for the rapid diagnosis of brain tumors at time of the surgery [7]. The method was providing reliable in the past with an accuracy rate of 95% [7]. This large retrospective analysis of smears in neurosurgical practice highlights the usefulness of this technique. It is a simple, reproducible and reliable technique which gives good cytological detail for making reasonably accurate diagnosis of lesions of CNS. Various authors used different stains like haematoxylin and eosin and May-Grunwald–Giemsa, and Papnicolaou stains [7-10]. The reported diagnostic accuracy of cytological smears ranged from 75% to 94% in various series [7-10]. To conclude, squash smear technique is a very accurate and rapid method of intraoperative diagnosis, but adequate clinical history, neuroimaging details and the intraoperative impression of the neurosurgeon if provided, helps the neuropathologists to improve the diagnostic accuracy [7,8]. Our reported diagnostic accuracy in chordomas was from 70%.

Clinical history, location and imaging help in looking for specific features. Smear technique is a rapid diagnostic method and interpretation is based on small sample of tissue. Inadequate clinical and imaging data can contribute to wrong diagnosis. The diagnostic accuracy was highest in tumours. However, partial correlation was due to grades and mixed tumours. The tumours which required more tissue, special stains and/or immunohistochemical confirmation for final diagnosis posed problems for diagnosis on smear [5,6]. We belief that our results are supportive of the accuracy of the procedure and comparable to other reported series.

Clinical prerequisites for a cytological diagnosis of brain tumors included; age of the patients, tumor location, and tumor size. The indications for a fine needle aspirations included midline lesions that are inaccessible to direct surgical remove or tend to infiltrate adjacent vital structures [7-9].

Crushed smears as well needle biopsy have been reported a pitfall in diagnosis included assessment of non-specific gliosis, necrosis and notochord cells in this case. Cytology of smears and crush preparation also offer some advantages to frozen sections; better preservation of cellular detail and minimizes the adverse changes as necrosis. The disvantages are the loss of tumor arquitecture detail in crush smears.

The classic chordoma consisted of multiple lobules that were separated by thin fibrous septa and that showed cords or strands of atypical physaliferous cells set within an abundant myxoid matrix [9]. Metachromatic stroma in between large physaliferous cells containing bubbly, vacuolated cytoplasm and small round nucleus. In contrast, the benign lesions consisted of intraosseous sheets of bland physaliferous cells without any extracellular matrix [6-9].

The cytologic features of chondroid chordoma observed in intraoperative crush and touch cytology revealed round or stellate cells distributed in a mucoid background without a typical epithelial cordlike arrangement [7-10]. The cytologic features of classical chordoma include conspicuous extracellular matrix in the background. Polygonal cells dissociated and arranged in small groups, were identified in all cases [10]. Stellate and cuboidal cells often contained intracytoplasmic vacuoles of varying sizes and round or oval nuclei and showed slight cellular pleomorphism [10]. Intranuclear inclusions, mitotic figures, and anisonucleosis were prominent features of some cases [10]. Physaliferous cells were also prominently found in these cases. In addition, the case with anaplastic features showed very bizarre cells with profound multinucleation and the presence of intranuclear cytoplasmic inclusions [6,7]. May Giemsa stain demonstrated the mucoid matrix and vacuolated cytoplasm of the tumor cells [8,9] additionally, crush preparations were effective in demonstrating well-differentiated chondroid elements [8,9]. Some case can presented some discrepancies between cytological features and definitive histological diagnoses, in some case immunohistochemistry could be a fully help.

Immunohistochemistry demonstrated cytoplasmic staining for low *vs* high-molecular-weight cytokeratins, vimentin, and epithelial membrane antigen, while glial fibrillary acidic protein and carcinoembryonic antigen have been reported as negative [10]. Immunocytochemistry with positivity for S-100 protein and cytokeratins have been used an essential adjunct in the cytologic diagnosis of chordoma and helped in distinguishing it from other chondrogenic tumors [7-9].

We must considered differential diagnosis, especially tumors with clear or vacuolated cells (clear cells meningioma, clear cell ependymoma, haemangioblastoma liposarcoma, lipoma, metastatic adenocarcinoma, and renal carcinoma), tumors with myxoid stroma (metastatic adenocarcinoma, myxoid liposarcoma, chondrosarcoma) and tumor with chondroid differentiation (chondrosarcoma, parachordoma, enchondroma). Thus, in cerebral localization based primarily affecting cranial as well as in sacrococcygeal region. The microscopic hallmark of these tumors is the presence of characteristic large cells with numerous cytoplasmic vacuoles known as physaliferous (Greek: droplet bearing) cells [7-9].

Metastases of the neoplasm may occur in 10-40% of the cases. Because of its unusual frequency, the diagnosis of chordoma may be difficult to render, especially on fine-needle aspiration biopsy. This distinction in the case of metastases can be made easily, where correlation of previous histology has been done and/or ancillary studies have been performed [10,11]. The presence of classic physaliferous cells on fine needle aspiration is diagnostic of chordoma, even in metastatic lesions [10-15]. Cellular chordomas can appear epithelioid in the sacrum and they may resemble metastatic squamous or transitional cell carcinomas. This distinction in the case of metastases can be made easily, where correlation of previous histology has been done and/or ancillary studies have been performed.

Appropriate immunocytochemical studies with clinical and cytological evaluation is recommended for avoiding misinterpretation with adenocarcinoma. Nuclear immunoreactivity of chordomas for S-100 protein appears to be a significant immunomarker for the differential diagnosis, although some adenocarcinomas may also be immunoreactive for S-100 protein [8-15].

Haemangioblastoma is a rare benign vascular tumor commonly seen in the cerebellum. There is a striking histologic similarity between cellular variant of haemangioblastoma and metastatic renal cell carcinoma [16], both tumors showed clear and vacuolated cells and diagnose can be missed due to these morphological similarities [16].

Liposarcomas (LS) smears are composed in different proportions of round, spindle cells, lipoblasts, and myxoid and vascular arborizing structures. Pure well-differentiated LS were frequently composed of lipoblasts and round or spindle cells were occasionally seen [17]. Dedifferentiated and sclerosing liposarcomas were composed of spindle or round cells, but lipoblasts were also occasionally present [17]. Myxoid or vascular arborizing structures were absent. Myxoid LS (including round and spindle cell LS) frequently showed a myxoid background and less frequently vascular arborizing structures. Tumor cells were round or spindle; lipoblasts were also seen [7-10]. Well-differentiated LS should be distinguished from hibernoma and spindle cell lipoma, and myxoid LS from myxoma, myxoid chondrosarcoma, chordoma, myxoid leiomyosarcoma, and myxoid malignant fibrous histiocytomas [10,17].

The presence of myxoid material in neoplasms should be included extraskeletal myxoid chondrosarcoma, chordoma, myxoid adenocarcinoma, myxoma, lipomatous tumors, nerve sheath tumors, smooth muscle tumors, gastrointestinal stromal tumor and other sarcomas [6-10].

Recognition of the cytological features characteristic may allow the distinction to be made on fine needle aspiration biopsy. Because of the prominent mucinous elements and papillary fronds, myxopapillary ependymoma may mimic other myxoid or papillary tumors cytologically [5-7,18,19]. This helps to distinguishing it from other chondrogenic tumors and metastatic mucous-producing carcinoma [6-9]. Exfoliative cytology of the sputum showed cohesive, epithelioid clusters composed of pale-stained, broad cytoplasm with a lacelike pattern, minimal nuclear atypism with anisonucleosis and characteristic mucoid substances [7-10].

Chondoid meningioma is a rare variant of meningioma that bears a striking histological resemblance to chordoma and has greater likelihood of recurrence [20]. Tumor is composed of cords and nests of eosinophilic vacuolated cells embedded in a myxoid matrix. A typical meningiomatous pattern was observed focally, and positive staining of the tumor cells for vimentin and epithelial membrane antigen confirmed the diagnosis of chordoid meningiomas [7-10]. GPAF is positive and useful in chondroid gliomas [20].

The cytologic criteria for differentiating chordoma from chondrosarcoma, mucinous metastatic adenocarcinoma and myxopapillary ependymoma include the vacuolated cells and the presence of bland nuclear features [21].

The cytomorphological features of Rhabdomyosarcoma (RMS) include small round to ovoid cells, and occasionally pleomorphic cells, with myxoid stroma, [20] typical rhabdomyoblastic cells and a myxoid background may be present in cases of embryonal RMS. However, in most cases studied; there are no specific cytological features [20-22].

The differential diagnosis of mass lesions of the sacrococcygeal region is broad and includes both benign and malignant neoplasms. Myxopapillary ependymoma is a variant of ependymoma that usually occurs in the sacrococcygeal region. Histologically, it is characterized by arborizing papillary fronds of capillaries with mucinous stroma rimmed by ependymal cells [20-22].

Cytology showed “fernlike” papillae and globules of mucinous and myxoid substance containing central capillaries. These structures were rimmed by one to several layers of mitotically inactive, mildly pleomorphic cuboidal to columnar cells with occasional pseudo nuclear cytoplasmic inclusions [21].

Some of these ependymal cells sent fibrillary processes toward the capillaries, suggestive of perivascular pseudorosettes [21]. There were also numerous isolated tumor cells and myxoid and chondroid material in the background [20,21].

The diagnosis of chordoma may be difficult to render, especially on Fine-Needle Aspiration Biopsy (FNAB) and crush smears. The cytological features of classical chordoma include conspicuous extracellular matrix in the background. Polygonal cells dissociated and in small groups, were identified in all cases. Physalipherous cells were also prominently found in these cases. In addition, the case with anaplastic features showed very bizarre cells with profound multinucleation and the presence of intranuclear cytoplasmic inclusions in chondroid type.

CONCLUSION

However, a clear-cut distinction of chordoma from other neoplasms is of utmost importance, since the prognosis and treatment of the patient will depend on the final diagnosis. In our cases, there were no differences between cranial *vs* sacrum location, however, chordoid chordoma showed atypia in Physalipherous cells than classic type. Maybe can say that classic chordoma showed vacuolar cells and chondroid type showed atypical and eosinophilic Physalipherous cells.

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Cite this article

Tena-Suck ML, Estrada-Natoli L, Kramis-Holland M, Corona-Cobian LE (2015) Chordomas; Crush Intraoperative Analysis. Ann Clin Cytol Pathol 1(2): 1006.