ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

Cannibalism in a benign soft tissue tumor (giant-cell tumor of the tendon sheath, localized type): a study of 66 cases

A. FERNANDEZ-FLORES

Department of Pathology,
Hospital El Bierzo, Ponferrada, Spain

Abstract

Cellular cannibalism refers to a phenomenon where a living cell is phagocytosed into a tumoral cell, where it eventually dies. With the exception of cells in suspension, cellular cannibalism has only been observed with malignant tumors. The finding of occasional images of cannibalism in our daily biopsies of giant cell tumors of the tendon sheath led us to examine this phenomenon further in a retrospective study of 66 cases from our archives. In each case, four morphological features were evaluated: evidence of giant cells, cannibalism, xanthomatous cells, and hemosiderin deposits. Five cases were randomly selected for further immunohistochemical study with the following antibodies: CD68, vimentin, leukocytary common antigen (LCA), Bcl-2 oncoprotein, p53, caspase-3, and Bax. Patients included 35 (53.03%) females and 31 (46.97%) males. Mean age was 50.73 years (range from 14 to 75 years). Giant cells were found in all cases but one (98.48%). Cannibalism was found in 56 cases (84.34%) and this phenomenon was graded as 1 in 35 cases, 2 in 13 cases, and 3 in six cases. The internalized cells frequently appeared apoptotic. Immunohistochemical analysis revealed that the internalized cells as well as the cannibal cells expressed CD68.

Keywords: cannibalism, entosis, emperipolesis, phagocytosis, autophagia.

Introduction

Cellular cannibalism is a phenomenon where a living cell is phagocytosed into a tumoral cell, where it eventually dies. The role of this process is not fully understood: it may function as a way of eliminating malignant cells or alternatively the ingested cell may serve as a source of nutrients for the proliferating cell that shows this cannibalistic behavior [1]. With the exception of certain phenomena seen in cells in suspension, [1–3] cellular cannibalism has only been associated with malignant tumors [4]. Cannibalism has even been hypothesized to be related to the metastatic capabilities of malignant cells [5].

We have not found any description in the available literature of cannibalism in a benign tumor of soft tissue. However, our observation of occasional images of cannibalism in our daily biopsies of benign giant cell tumors of the tendon sheath prompted us to examine this phenomenon further in a retrospective study of material from our archives.

Materials and Methods

We recovered from our archives the cases of localized type of giant cell tumor of the tendon sheath that had been diagnosed in the last ten years. In each case, we took note of the following information: age, gender, and size of the tumor. All cases were also reviewed to confirm the histopathological diagnosis. In each case, the following four morphological features were evaluated: evidence of giant cells, cannibalism, xanthomatous cells, and hemosiderin deposits. Each feature was quantified on a scale from 0 to 4, according to the parameters described in Table 1.

Table 1 – ***Morphologic features that were evaluated in the biopsies of giant cell tumor of tendon sheath***

Values	Giant cells	Cannibalism	Xanthomatous cells	Hemosiderin
0	Not evidenced.	Not evidenced.	Not evidenced.	Not evidenced.
1	Occasional and scattered.	Less than 10% of the giant cells showing cannibalism.	Xanthic areas represent less than 5% of the tumor.	Less than 5% of the tumor with hemosiderin deposits.
2	Seen in up to 1/10 HPFs.	10 to <25% of giant cells showing cannibalism.	Xanthic areas represent 5% to 15% of the tumor.	≥ 5% to 15% of the tumor with hemosiderin deposits.
3	Seen in more than 1/10 HPFs but in less than 5/10 HPFs.	25–90% of giant cells showing cannibalism.	Xanthic areas represent 5% to 15% of the tumor.	>15% of the tumor to 30% of the tumor with hemosiderin deposits.
4	Seen in more than half of the HPFs.	More than 90% of the giant cells showing cannibalism.	More than 50% of the tumor showing xanthic areas.	More than 30% of the tumor showing hemosiderin deposits.

HPF: High power field (400×).

Hemosiderin deposits were confirmed with Perls histochemical stain.

Five cases were randomly selected to perform further immunohistochemical analyses using the following antibodies: CD68 (Dako, clone PG-M1, isotype IgG3 kappa, code M0876); vimentin (Dako, clone V9, isotype IgG1 kappa, clone M0725); leukocytary common antigen (LCA) (Dako, clone 2B11 + PD7/26, isotype IgG1, kappa + IgG1, kappa, code M0701), Bcl-2 oncoprotein (Dako, clone 124, isotype IgG1, kappa, code M0887), p53 (Dako, clone 318-6-11, code M3629), caspase-3 (Novocastra, NCL CPP32), and Bax (Dako, code A3533).

Results

We retrieved 66 cases from our archives. Table 2 shows the morphological features studied in the biopsies. Patients included 35 females (53.03%) and 31 males (46.97%), with a mean age of 50.73 years (range from 14 to 75 years). Figure 1 shows the percentages of cases that showed various morphological features.

Table 2 – ***Cases studied; clinical and morphologic findings***

Case No.	Gender	Age [years]	Size of the tumor [cm]	Giant cells	Cannibalism	Hemosiderin deposits	Xanthomatous cells
1.	F	60	1	1	1	1	1
2.	F	58	1.3	1	0	0	0
3.	F	42	1.5	4	3	2	1
4.	M	40	1.7	2	2	2	1
5.	F	40	2	1	1	3	2
6.	F	24	1.4	3	2	1	1
7.	M	56	1	3	2	2	1
8.	M	32	1.5	1	0	2	1
9.	M	74	1.7	1	1	1	3
10.	M	29	1.3	3	2	1	1
11.	F	14	1	3	2	1	3
12.	F	53	1.4	3	2	2	1
13.	F	54	1.4	3	3	1	0
14.	F	65	1.7	2	1	1	2
15.	M	18	2	2	1	3	1
16.	M	45	0.5	3	1	0	0
17.	M	25	0.8	3	1	3	1
18.	M	45	2.3	3	3	2	1
19.	F	45	0.8	1	1	1	1
20.	M	43	1.5	3	2	1	1
21.	M	46	1.4	3	1	2	1
22.	M	71	2.3	1	1	1	4
23.	M	58	1.3	2	1	1	2
24.	M	65	1.6	2	1	1	1
25.	F	57	1.3	2	1	1	1
26.	M	67	1.1	1	1	0	0
27.	F	44	1.8	1	0	1	3
28.	F	62	1.5	1	1	1	3
29.	F	53	1.3	2	1	3	1
30.	M	47	0.5	4	4	2	1
31.	M	47	0.7	2	2	1	0
32.	M	53	2	3	1	1	1
33.	F	69	1.8	1	1	2	1
34.	M	54	0.4	1	1	0	0
35.	M	30	1	1	1	2	1
36.	F	20	0.7	1	0	2	0
37.	M	31	1	0	1	0	0
38.	F	66	1.3	1	0	1	0
39.	F	24	0.8	1	0	0	0
40.	F	71	2.1	1	1	1	1
41.	F	53	1	3	1	2	1

Case No.	Gender	Age [years]	Size of the tumor [cm]	Giant cells	Cannibalism	Hemosiderin deposits	Xanthomatous cells
42.	M	28	2.5	1	1	2	2
43.	F	58	0.8	1	0	0	1
44.	F	70	1.9	2	1	1	4
45.	F	75	1.3	1	1	2	1
46.	F	67	1.8	3	2	1	1
47.	M	59	1.5	2	1	2	1
48.	M	60	1	3	2	1	0
49.	F	34	0,9	2	1	1	1
50.	F	59	1.5	1	1	2	1
51.	F	68	2	1	1	3	3
52.	F	70	1.5	2	1	2	1
53.	F	71	0.8	2	2	2	1
54.	M	42	1.5	1	1	3	1
55.	F	67	1	1	0	0	0
56.	M	56	1	1	0	1	0
57.	F	66	2	1	1	2	1
58.	F	58	0.8	1	0	0	1
59.	F	59	2	2	2	2	1
60.	M	58	2.7	1	0	0	3
61.	F	43	1.7	1	2	1	2
62.	F	62	2.5	3	3	2	2
63.	M	34	2	1	1	1	1
64.	M	17	1	3	3	1	2
65.	M	57	3	4	3	1	3
66.	M	60	1.5	2	1	1	3

F: Female; M: Male.

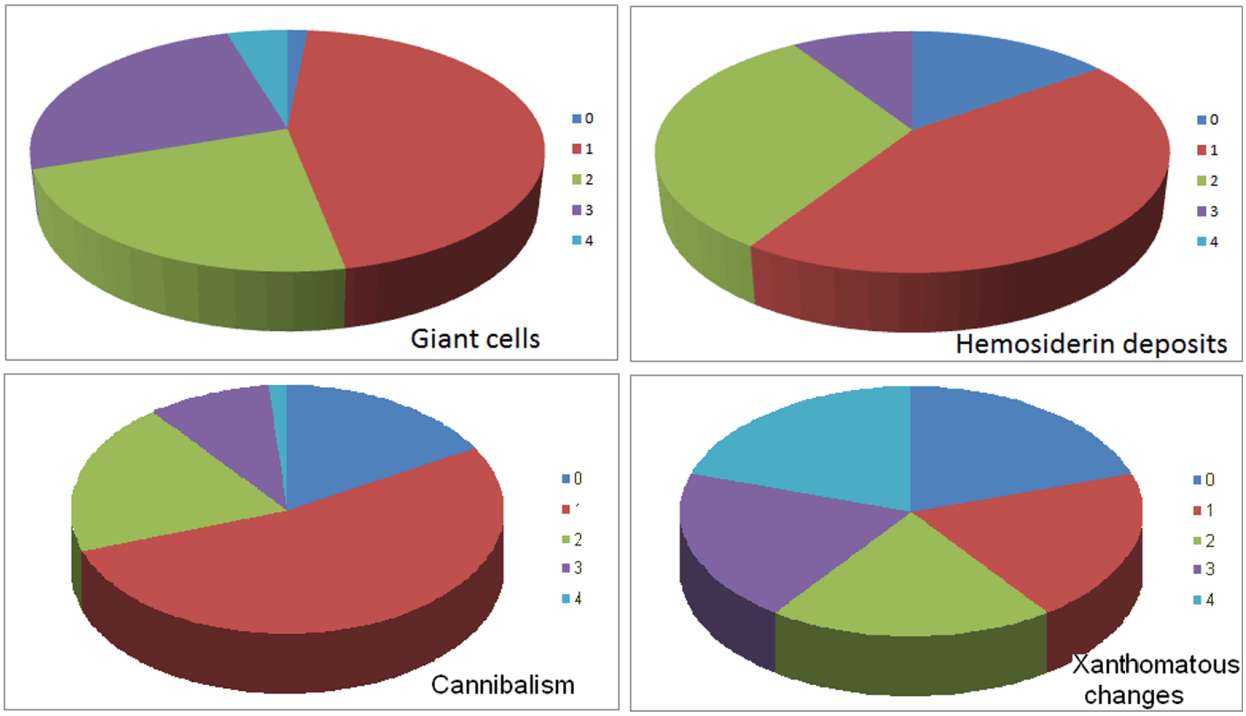

Figure 1 – ***The scheme shows the four morphologic features that were investigated. They were graded from 0 to 4 and the diagrams show the percentages of cases belonging to each grade per feature.***

We found giant cells in all cases but one (98.48%). Cannibalism was found in 56 cases (84.34%) and was graded as 1 in 35 cases, 2 in 13 cases, and 3 in six cases. The cannibal cell was not always multinucleated and on occasion, we saw a uninucleate cell cannibalizing another cell (Figure 2, top and middle). In some cases, the cannibal cell had just two or three nuclei, but this was a small cell (Figure 2, bottom), while in most occasions,

they were multinucleated large cells (Figure 3, top). Cannibalism of two cells (Figure 3, middle) or of several cells (Figure 3 bottom) was also seen.

The internalized cells frequently displayed an apoptotic appearance when stained with Hematoxylin–Eosin, with loss of the nucleus and an increase in cytoplasmic density (Figure 4). Immunohistochemistry analysis revealed that the internalized cells as well as the cannibal cells expressed CD68 (granular cytoplasmic expression) (Figure 5). The surviving internalized cells did not express the apoptotic markers Bax or p53. In contrast, expression of caspase-3 was evident occasionally in the internalized cells (Figure 6, top). No immunoexpression of Bcl-2 was found for the internalized cells, which contrasted with the diffuse, mild cytoplasmic immunostaining found in the multinucleate cells (Figure 6, bottom).

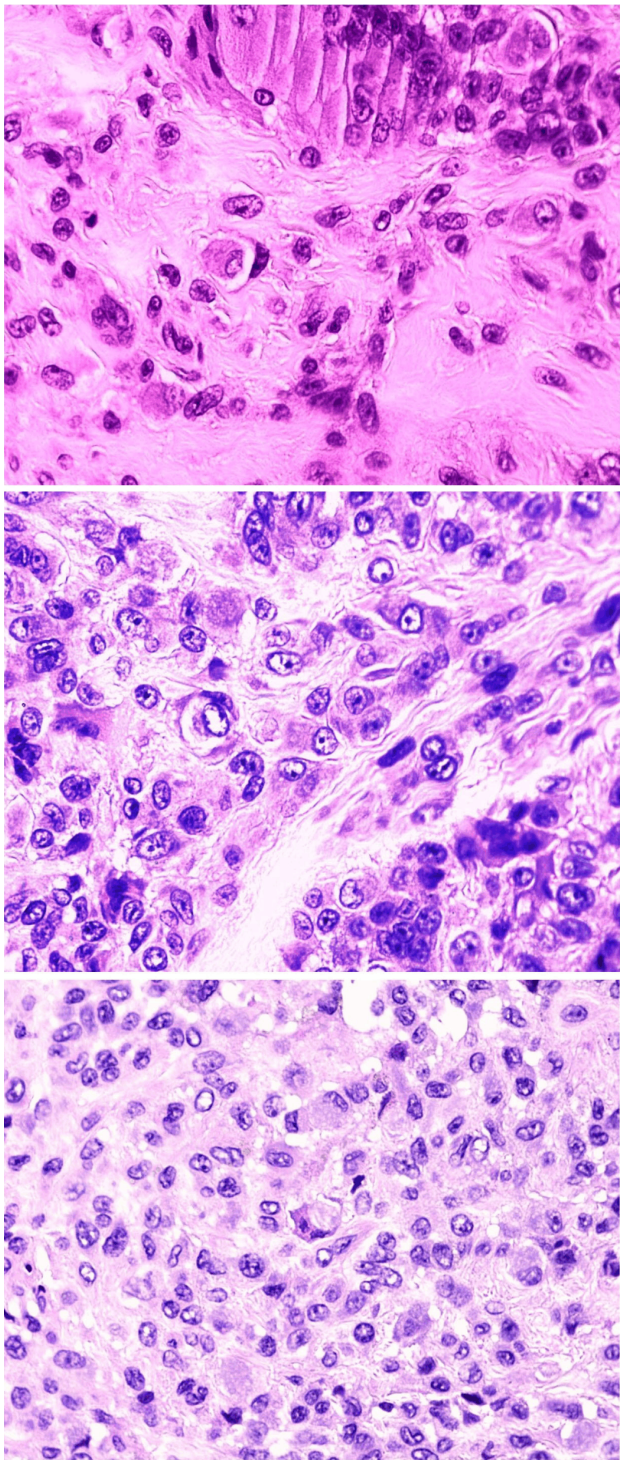

Figure 2 – ***Sometimes, one cell with only one nucleus, was seen engulfing another cell (top and middle), and some other times a small cell with two or three nuclei, was engulfing another cell (ob. ×20).***

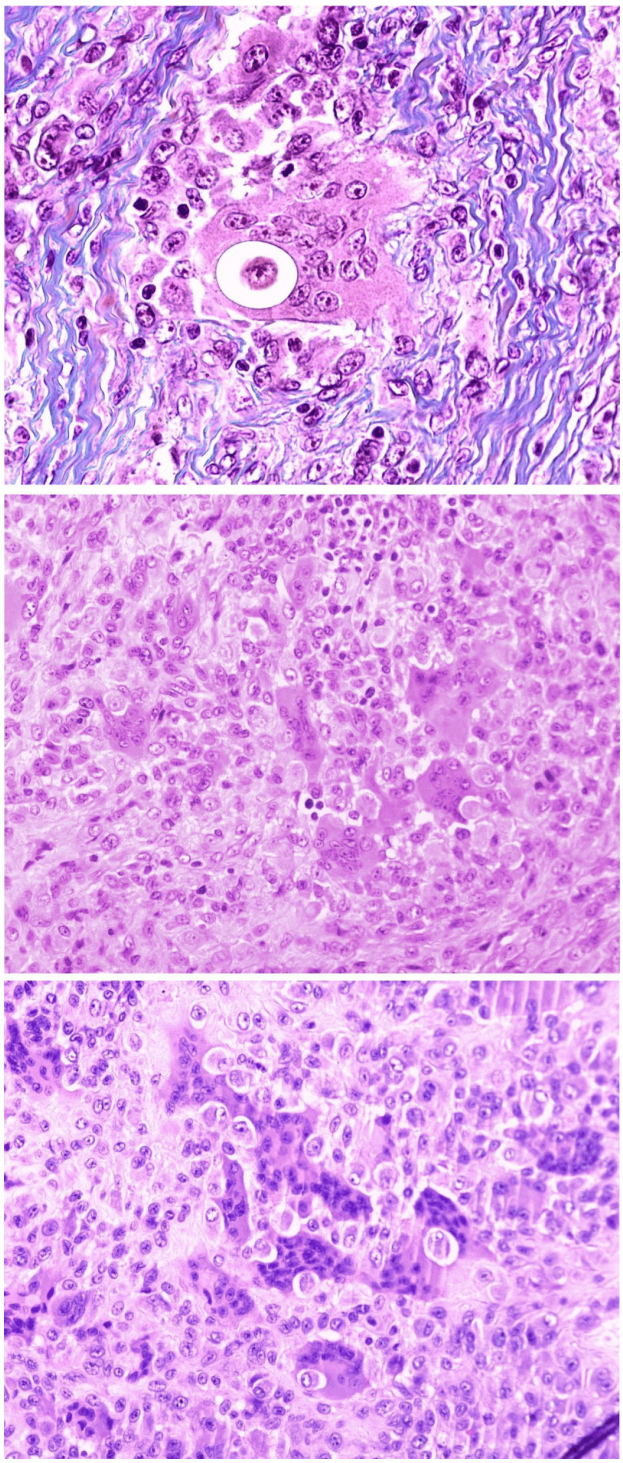

Figure 3 – ***In most cases, the cell engulfing others had many nuclei (top, ob. ×20). Sometimes, these large cells could be seen engulfing two (middle, ob. ×10) or several cells (bottom, ob. ×10).***

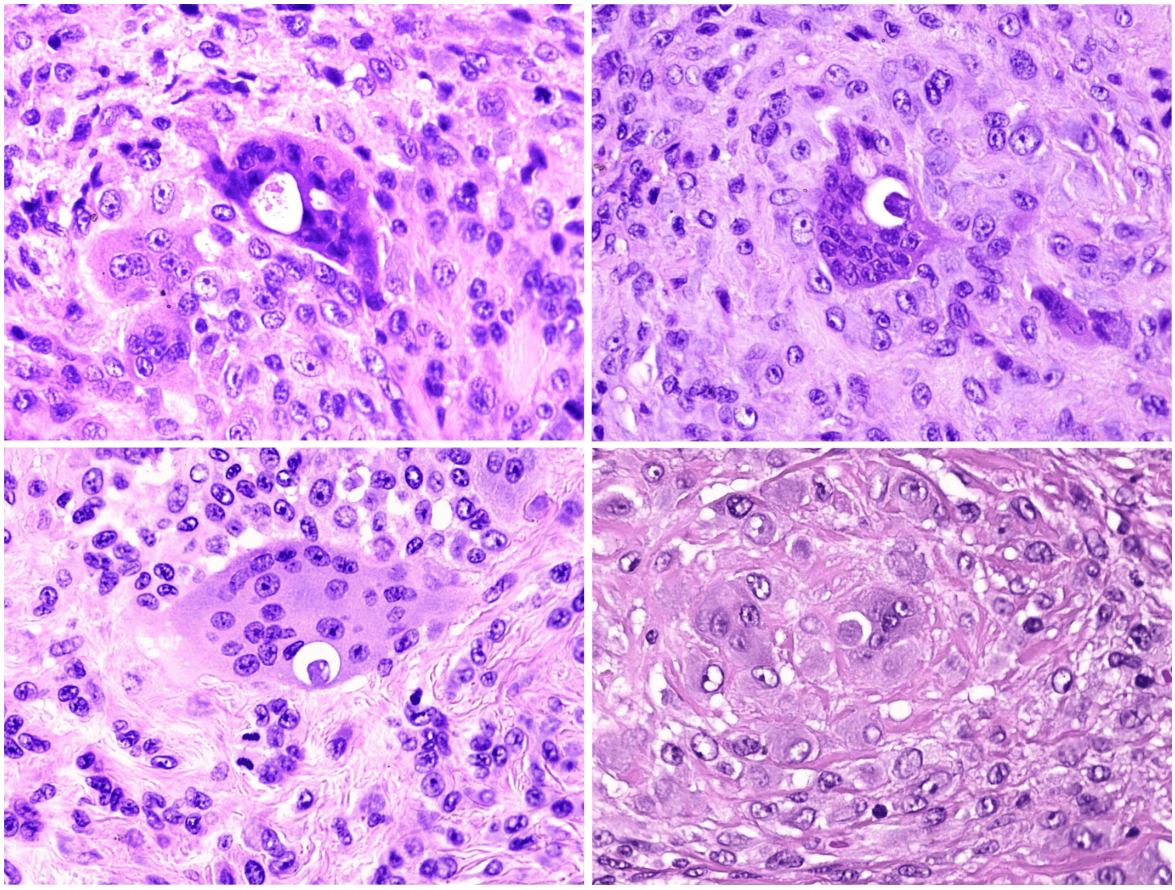

Figure 4 – ***Several images in which the internalized cell shows an apoptotic appearance in the routine staining, with condensed and corrugated cytoplasm and no nucleus (ob. ×20).***

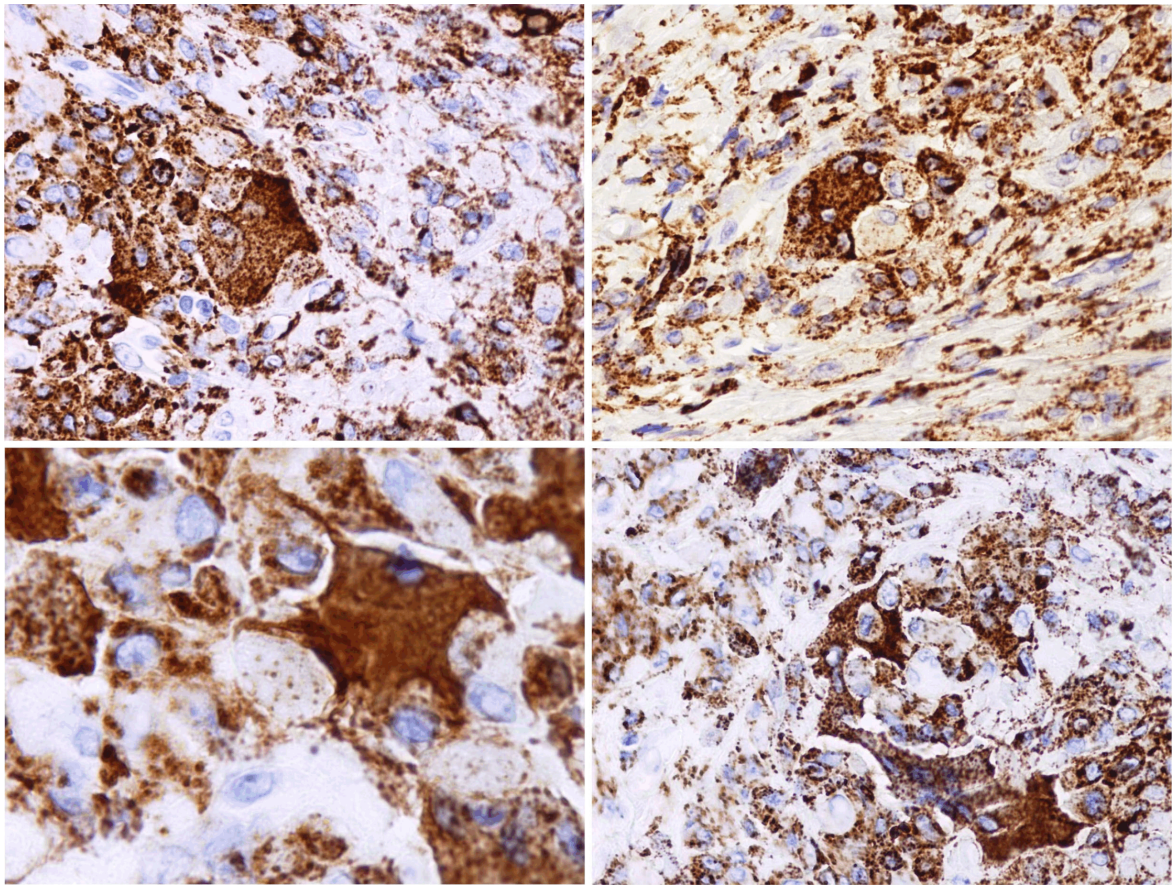

Figure 5 – ***The internalized cells as well as the cannibal cells showed cytoplasmic expression of CD68 (top left and right, and bottom right: ob. ×20; bottom left: ob. ×40).***

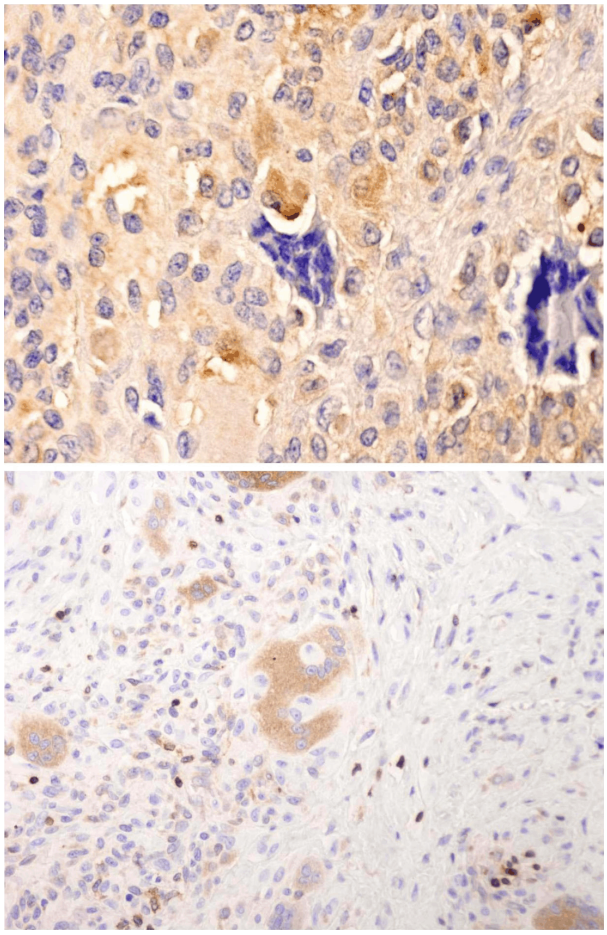

Figure 6 – ***Some of the internalized cells showed immunoexpression of caspase-3 (top, ob. ×20). No expression on the engulfed cells was seen with Bcl-2 (bottom, ob. ×10) (the multinucleated cell showed cytoplasmic mild positivity).***

Discussion

Cell cannibalism (from the Spanish "cannibal", due to the anthropophagic practices from the past in the Caribbean Sea) is defined as "a large cell enclosing a slightly smaller one within its cytoplasm" [4]. It was first described by Leyden, in 1904, who named these cells "bird's-eye cells". The engulfed cell is still alive when internalized, but the process implies its death. In this sense, this differs from several other types of cell engulfment (Table 3).

Table 3 – ***Types of cellular internalization as described in literature***

Emperipolesis [32]	▪ The engulfed cells are hematopoietic; ▪ The cells are only temporarily internalized; ▪ The internalized cells are not destroyed.
Phagocytosis [10]	▪ The engulfing cells are macrophages; ▪ The engulfed cells are death; ▪ The macrophage internalizes the other cell with pseudopodes.
Autophagy [33]	▪ The cell engulges damaged organelles through autophagosomes.
Entosis [1]	▪ Cell-in-cell invasion process.

Cannibalism has been described as being an exclusive property of malignant tumor cells [4]. For instance, it has been described in carcinoma of the lung [6, 7] endometrial stromal sarcoma [8], gastric adenocarcinomas [9], malignant melanoma [10] and, in general, in many malignant tumors in advanced stages [7, 11]. Some investigators have also demonstrated that melanoma cell lines derived from metastatic lesions exhibit phagocytosis, whereas primary tumors do not [12]. This is why some researchers have hypothesized that cannibalism could be used as a marker to indicate the metastatic potential of the tumor cells [5].

A form of "benign cannibalism" has been described by Ohsaki H *et al.* in urine samples of patients with renal glomerular disease [3]. Recently, a process known as "entosis" has also been described in benign as well as malignant cellular lines in suspension [2]. This is a process of cell-in-cell invasion that seems to play a physiological role in the elimination of cells detached from a surface, as in the clearance of some luminae [1]. However, from these examples, we were unable to find any description in the literature of cannibalism in a benign tumor.

The giant cell tumor of the tendon sheath is a benign lesion with two main forms of presentation: localized (known in the past as nodular tenosynovitis) and diffuse. The localized form is more common on the hands [13] but it can also be seen on feet, knees and ankles [14]. This tumor commonly presents as a lobulated, nontender, slow-growing mass.

There was some debate in the pass as to whether the giant cell tumor of the tendon sheath is a tumor or a reactive condition [15–19]. Polymerase chain reaction (PCR) assays for methylation of the X-linked human androgen receptor (HUMARA) in female patients have shown that these masses are polyclonal proliferations [20]. However, some lesions have shown aneuploidy [21] and some have presented clonal chromosomal abnormalities [22]. More recently, it was demonstrated that they are neoplasms, which harbor rearrangement of chromosome 1p13 with overexpression of CSF1, which drives the influx of non-neoplastic inflammatory cells (mainly histiocytes) in these tumors [23].

In classic texts, images of cell internalization, similar to the ones we have presented here, are frequently shown, but curiously, they are not described or mentioned in the text [24]. However, mention is commonly made that giant cells originate by fusion of mononuclear cells [24]. Therefore, the images of cellular internalization that we present in the current report might arguably be related to the formation of giant cells from mononuclear cells. Ultrastructural studies on giant cell tumors of the tendon sheath by Anazawa U *et al.* revealed how a few mononuclear cells showed cell-to-cell contact [25]. This contact had previously been described by Alguacil-Garcia A *et al.* [26]. Athanasou NA *et al.* also described in their ultrastructural study how giant cells "were admixed with 'mononuclear' cells" [27]. Hosaka M *et al.* studied a model of formation of giant cells that involved fusion of cells derived from a human giant cell tumor of tendon sheath [28]. Using an *in vitro* fluorescent cell membrane labeling technique, they demonstrated a mosaic of green and red colors in giant cells, indicating cell membrane fusion [28]. However, they did not describe images of cellular internalization

or cannibalism, nor is this type of phenomenon evident in any of their figures [28].

In contrast, we observed internalized cells with clear signs of apoptosis. How these apoptotic nuclei could survive after cellular fusion and subsequently contribute to the formation of a multinucleate cell is difficult to imagine. For this reason, we have investigated the expression of some immunohistochemical markers associated with apoptosis, specifically Bcl-2, Bax, p53, and caspase-3 in the internalized cells. Bax and Bcl-2 belong to a family of proteins that is involved in the regulation of apoptosis. Overexpression of Bax promotes cell death, whereas Bcl-2 shows an antiapoptotic effect, due to its ability to form a heterodimer with Bax. This type of antiapoptotic effect is especially efficient when Bcl-2 is over-expressed [29]. We also studied p53, because in several cellular types (lymphocytes, for instance), over-expression of p53 leads to apoptosis [30]. Lastly, caspases are crucial mediators of apoptosis. Among these, caspase-3 is a frequently activated death protease that catalyzes the specific cleavage of many key cellular proteins [31]. When we looked for these apoptosis markers in the internalized cells, we found expression of at least caspase-3, as well as lack of expression of Bcl-2.

The cannibal cells, as well as the internalized cells, expressed CD68, which is a reflection of their histiocytic nature. We used the antibody from the clone PG-M1 (phosphoglucomutase 1) because it labels human monocytes and macrophages, but not myeloid cells. No overlap occurs between this marker and fibrohistiocytic markers [32].

Conclusions

We have shown images of cannibalism in a benign condition, namely the localized type of giant-cell tumor of the tendon sheath, which we believe is the first such report published. We also have demonstrated that the internalized cells, as well as the cannibal cells, expressed histiocytic markers. Lastly, we have shown how internalized cells express some markers that suggest that the internalization induces the apoptotic death of the internalized cell.

References

[1] White E, *Entosis: it's a cell-eat-cell world*, Cell, 2007, 131(5):840–842.

[2] Overholtzer M, Mailleux AA, Mouneimne G, Normand G, Schnitt SJ, King RW, Cibas ES, Brugge JS, *A nonapoptotic cell death process, entosis, that occurs by cell-in-cell invasion*, Cell, 2007, 131(5):966–979.

[3] Ohsaki H, Haba R, Matsunaga T, Nakamura M, Kiyomoto H, Hirakawa E, *'Cannibalism' (cell phagocytosis) does not differentiate reactive renal tubular cells from urothelial carcinoma cells*, Cytopathology, 2009, 20(4):224–230.

[4] Sharma N, Dey P, *Cell cannibalism and cancer*, Diagn Cytopathol, 2011, 39(3):229–233.

[5] Chandrasoma P, *Polymorph phagocytosis by cancer cells in an endometrial adenoacanthoma*, Cancer, 1980, 45(9):2348–2351.

[6] Craig ID, Desrosiers P, Lefcoe MS, *Giant-cell carcinoma of the lung. A cytologic study*, Acta Cytol, 1983, 27(3):293–298.

[7] DeSimone PA, East R, Powell RD Jr, *Phagocytic tumor cell activity in oat cell carcinoma of the lung*, Hum Pathol, 1980, 11(5 Suppl):535–539.

[8] Hong IS, *The exfoliative cytology of endometrial stromal sarcoma in peritoneal fluid*, Acta Cytol, 1981, 25(3):277–281.

[9] Caruso RA, Muda AO, Bersiga A, Rigoli L, Inferrera C, *Morphological evidence of neutrophil-tumor cell phagocytosis (cannibalism) in human gastric adenocarcinomas*, Ultrastruct Pathol, 2002, 26(5):315–321.

[10] Fais S, *Cannibalism: a way to feed on metastatic tumors*, Cancer Lett, 2007, 258(2):155–164.

[11] Abodief WT, Dey P, Al-Hattab O, *Cell cannibalism in ductal carcinoma of breast*, Cytopathology, 2006, 17(5):304–305.

[12] Guo KJ, Yamaguchi K, Enjoji M, *Undifferentiated carcinoma of the gallbladder. A clinicopathologic, histochemical, and immunohistochemical study of 21 patients with a poor prognosis*, Cancer, 1988, 61(9):1872–1879.

[13] Darwish FM, Haddad WH, *Giant cell tumour of tendon sheath: experience with 52 cases*, Singapore Med J, 2008, 49(11):879–882.

[14] Monaghan H, Salter DM, Al-Nafussi A, *Giant cell tumour of tendon sheath (localised nodular tenosynovitis): clinicopathological features of 71 cases*, J Clin Pathol, 2001, 54(5):404–407.

[15] Gehweiler JA, Wilson JW, *Diffuse biarticular pigmented villonodular synovitis*, Radiology, 1969, 93(4):845–851.

[16] Kahn LB, *Malignant giant cell tumor of the tendon sheath. Ultrastructural study and review of the literature*, Arch Pathol, 1973, 95(3):203–208.

[17] Coster AA, *Giant cell tumor of tendon sheath (benign synovioma)*, J Am Podiatry Assoc, 1976, 66(7):538–541.

[18] Castens HP, Howell RS, *Malignant giant cell tumor of tendon sheath*, Virchows Arch A Pathol Anat Histol, 1979, 382(2):237–243.

[19] Cavaliere A, Sidoni A, Bucciarelli E, *Giant cell tumor of tendon sheath: immunohistochemical study of 20 cases*, Tumori, 1997, 83(5):841–846.

[20] Vogrincic GS, O'Connell JX, Gilks CB, *Giant cell tumor of tendon sheath is a polyclonal cellular proliferation*, Hum Pathol, 1997, 28(7):815–819.

[21] Abdul-Karim FW, el-Naggar AK, Joyce MJ, Makley JT, Carter JR, *Diffuse and localized tenosynovial giant cell tumor and pigmented villonodular synovitis: a clinicopathologic and flow cytometric DNA analysis*, Hum Pathol, 1992, 23(7):729–735.

[22] Reilly KE, Stern PJ, Dale JA, *Recurrent giant cell tumors of the tendon sheath*, J Hand Surg Am, 1999, 24(6):1298–1302.

[23] West RB, Rubin BP, Miller MA, Subramanian S, Kaygusuz G, Montgomery K, Zhu S, Marinelli RJ, De Luca A, Downs-Kelly E, Goldblum JR, Corless CL, Brown PO, Gilks CB, Nielsen TO, Huntsman D, van de Rijn M, *A landscape effect in tenosynovial giant-cell tumor from activation of CSF1 expression by a translocation in a minority of tumor cells*, Proc Natl Acad Sci U S A, 2006, 103(3):690–695.

[24] Enzinger FM, Weiss SW, *Soft tissue tumors*, Mosby, St. Louis, 1983.

[25] Anazawa U, Hanaoka H, Shiraishi T, Morioka H, Morii T, Toyama Y, *Similarities between giant cell tumor of bone, giant cell tumor of tendon sheath, and pigmented villonodular synovitis concerning ultrastructural cytochemical features of multinucleated giant cells and mononuclear stromal cells*, Ultrastruct Pathol, 2006, 30(3):151–158.

[26] Alguacil-Garcia A, Unni KK, Goellner JR, *Giant cell tumor of tendon sheath and pigmented villonodular synovitis: an ultrastructural study*, Am J Clin Pathol, 1978, 69(1):6–17.

[27] Athanasou NA, Quinn J, Ferguson DJ, McGee JO, *Bone resorption by macrophage polykaryons of giant cell tumour of tendon sheath*, Br J Cancer, 1991, 63(4):527–533.

[28] Hosaka M, Hatori M, Smith R, Kokubun S, *Giant cell formation through fusion of cells derived from a human giant cell tumor of tendon sheath*, J Orthop Sci, 2004, 9(6):581–584.

[29] Reap EA, Felix NJ, Wolthusen PA, Kotzin BL, Cohen PL, Eisenberg RA, *Bcl-2 transgenic Lpr mice show profound enhancement of lymphadenopathy*, J Immunol, 1995, 155(11):5455–5462.
[30] Cory S, *Regulation of lymphocyte survival by the bcl-2 gene family*, Annu Rev Immunol, 1995, 13:513–543.
[31] Porter AG, Jänicke RU, *Emerging roles of caspase-3 in apoptosis*, Cell Death Differ, 1999, 6(2):99–104.
[32] Kunisch E, Fuhrmann R, Roth A, Winter R, Lungershausen W, Kinne RW, *Macrophage specificity of three anti-CD68 monoclonal antibodies (KP1, EBM11, and PGM1) widely used for immunohistochemistry and flow cytometry*, Ann Rheum Dis, 2004, 63(7):774–784.

Corresponding author
Angel Fernandez-Flores, MD, PhD, Servicio de Anatomía Patológica, Hospital El Bierzo, Medicos Sin Fronteras 7, 24411 Fuentesnuevas, Leon, Spain; Phone (00 34) 987 42 37 32, e-mail: gpyauflowerlion@terra.es

Received: January 2nd, 2012

Accepted: February 25th, 2012