International Orthopaedics (SICOT) (2000) 24:197–201

ORIGINAL PAPER

Michele Attilio Rosa · Marco Galli · Guido Fadda
Nicola Maggiano · Giovanni Francesco Gambino

Proliferating cell nuclear antigen labelling index in localised pigmented villo-nodular synovitis and its relationship to the size of nodules

Accepted: 29 February 2000

Abstract Proliferating cell nuclear antigen (PCNA) is one of the cell cycle-related proteins directly involved in DNA synthesis. It is a marker of cellular proliferation and has been shown to correlate with ploidy and proliferative activity of cells. Its expression has been used to estimate the growth fraction of human cancer and its prognostic value. Pigmented villo-nodular synovitis (PVNS) is characterised by a nodular lesion in the paratendinous synovial tissue or, less frequently, in a joint. Whether PVNS is a neoplastic or inflammatory lesion remains controversial. We have studied immunohistochemical PCNA expression with pc10 monoclonal antibody in 16 paraffin sections, in 16 cases of localised PVNS, or giant cell tumour of tendon sheath. We have found significant correlation between the size of the lesions and PCNA-LI (labelling index).

Résumé L'antigène nucleaire de prolifération cellulaire (PCNA) est une des proteines du cycle cellulaire et il est impliqué directement dans la synthèse du DNA. Il s'agit certainement d'un indicateur de prolifération cellulaire. Son expression a été employé pour évaluer l'index de prolifération de la tumeur humaine et son valeur au point de vue pronostique. La synovite villo-nodulaire pigmentée (PVNS) est caractérisée par une lésion nodulaire plus fréquente dans les localisations tendineuses que dans les articulations. S'il s'agit d'un processus néoplasique ou d'une lésion inflammatoire reste controversée. Nous avons étudiés l'expression immunohistochimique du PCNA avec l'anticorps monoclonal PC10 en 16 lames paraffinés relatives à 16 synovites villo-nodulaires pigmentées localisées ou tumeurs à cellules geantes des gaines tendineuses. Nous avons trouvés une corrélation significative entre les dimensions des lésions nodulaires et le PCNA labelling index (PCNA-LI).

M.A. Rosa · M. Galli · G.F. Gambino
Istituto di Clinica Ortopedica,
Università Cattolica del Sacro Cuore, Rome, Italy

G. Fadda · N. Maggiano
Istituto di Anatomia Patologica,
Università Cattolica del Sacro Cuore, Rome, Italy

M.A. Rosa (✉)
Largo Maccagno, 7, 00136, Rome, Italy
Tel: +39-6-35400582
e-mail: gfgambino@tiscalinet.it

Introduction

PCNA has been shown to correlate with ploidy and proliferative activity of cells [4, 13, 15, 19] and its expression has been used to estimate the growth fraction of malignant tumours in humans [1, 27]. PCNA immunostaining is a sensitive method which can easily be performed on paraffin-embedded specimens [1]. PVNS (Figs. 1–3) is a proliferative process involving the synovial membrane of joints, tendons and bursae [9]. The localised form is characterised by a nodular lesion in paratendinous synovial tissue or, less frequently, in a joint. These lesions may become painful after a long period of slow growth and may infrequently grow very rapidly; they are often associated with osteolytic lesions in the surrounding bone. A recurrence rate of 10–20% has been reported after excision.

Materials and methods

Sixteen cases of giant cell tumour of tendon sheath were retrieved from the files of the Istituto di Clinica Ortopedica and selected for study on the basis of their histologic features, the availability of blocks for immunohistochemical studies and photographic documentation of the gross specimens. All tissues were fixed in formalin and paraffin-embedded. Sixteen specimens from 16 patients (8 males, 8 females); with a mean age of 36 years (12–74 years) were included. Immunohistochemical staining was performed on sections from the paraffin-embedded tissue. Five-millimeter sections were deparaffinised in xylene and then rehydrated. Endogenous peroxidase activity was blocked with 1% H_2O_2 methanol. Following a short rinse in PBS (phosphate buffer solution), the sections were pre-incubated with 1% normal horse serum. PC10 mouse monoclonal antibodies (Ylem, Aq, Italy) to human PCNA were applied in a 1:50 dilution for 1 h. They were treated with the avidin biotin peroxidase complex (ABC-Elite Kit; Vector Laboratories, Burlingame, Calif., USA). The reaction products in the sections were visualised with freshly prepared 0.01% and 3.3-diaminobenzidine tetrahydrochloride (DAB; Sigma, USA) containing 0.02% H_2O_2. The sections

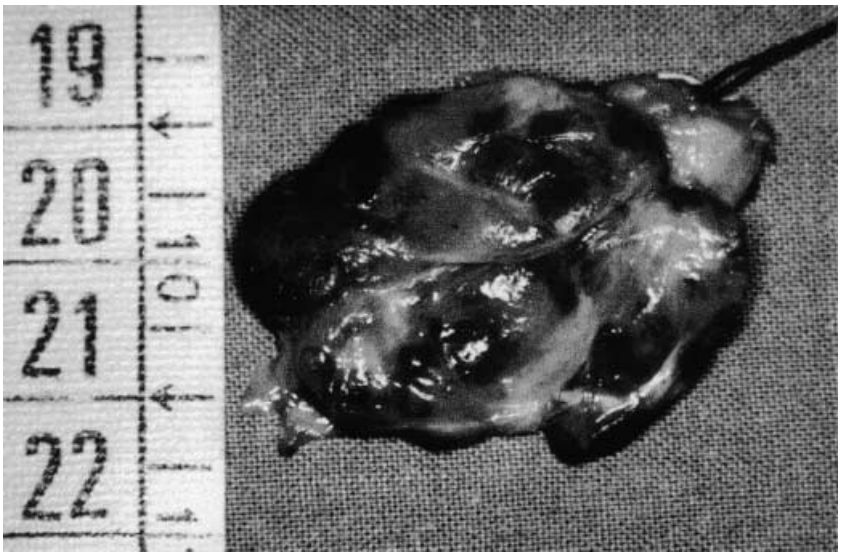

Fig. 1 Gross specimen of patient 12 longitudinally cut and opened

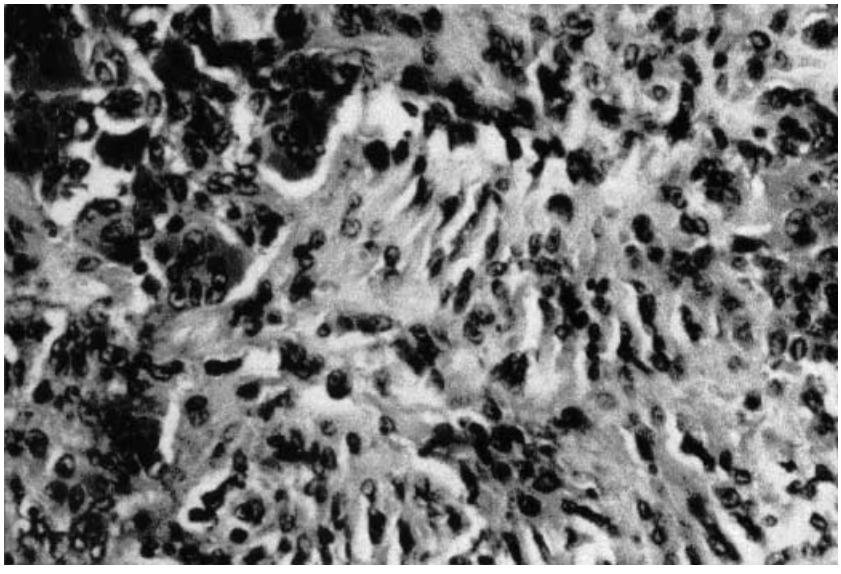

Fig. 2 Histological appearance of pigmented villonodular synovitis. H&E, ×400

were counterstained with Harris hematoxylin. PCNA immunoreactivity was quantified by selecting five different areas per histological section with the highest positivity and counting was recorded using ×400 magnification. Both labelled and unlabelled nuclei were counted and the PCNA-labelling index (PCNA-LI), expressed as a ratio of positively stained nuclei to total nuclei counted, was determined by counting 500–1000 nuclei. Two examiners (both authors) performed these counts twice at intervals of 2 weeks and calculated the mean. The dimension of the lesions was obtained by measuring the mean between the maximum and the minimum size on the photographic images of the gross specimen. Images of the specimen beside a graduated ruler had been taken immediately after excision.

Statistical analysis was performed using the Spearman rank correlation coefficient *r*.

Results

Immunohistochemical analyses show that giant cells do not stain positively with PCNA antibodies. The clinical findings and PCNA-LI are shown in Table 1. The mean PCNA-LI in our cases was 0.262 (standard deviation = 0.176).

A significant correlation was found between the size of the lesions and the PCNA-LI:Spearman r (corrected for ties) = 0.923; t=8.97, P<0.001.

Discussion

Whether the aetiology of PVNS is neoplastic or inflammatory remains controversial. Jaffe proposed the denomination of synovitis on the basis of hyperplastic stromal cells in a milieu of hyalinized collagen with a tendency to produce fibrosis [12]. The association between PVNS and trauma, or rheumatoid arthritis, has been reported [21]. Some attempts have been made to induce PVNS experimentally. Lesions have been induced by injecting colloidal iron into the joints of rabbits [32] and by injecting blood, colloidal iron, plasma and gum acacia into the ankles of monkeys [30]. The colloidal iron produced changes similar to PVNS except for the absence of foam

Table 1 Overview of cases studied

Case	Age (years)	Gender	Localization	Dimension (cm)	PCNA-LI
1	12	M	Thumb	0.8	0.05
2	66	F	IV finger	1	0.206
3	23	F	II finger	1	0.327
4	48	M	II finger	1	0.125
5	60	F	IV finger	1	0.182
6	16	F	II toe	1	0.07
7	21	M	II finger	1.2	0.02
8	25	M	III toe	1.2	0.312
9	33	M	II toe	1.5	0.206
10	36	M	V finger	1.7	0.224
11	74	F	Hand	2	0.253
12	27	F	III-IV inter-metatarsal space	2.5	0.137
13	36	F	III-IV inter-metatarsal space	3	0.509
14	24	M	Knee	3	0.552
15	50	F	Elbow	3.5	0.512
16	26	M	Post. tibial tendon sheath	4	0.511

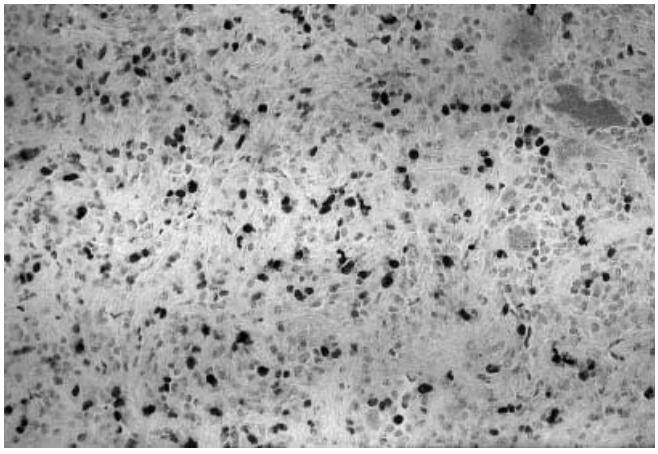

Fig. 3 Immunohistochemical staining with PC10 antibody (anti-PCNA). ×400

Table 2 Immunophenotypes of mononuclear cells (*PCNA* proliferating cell nuclear antigen, marker of cellular proliferation; *fibronectine* marker of fibroblastic cells; *CD68* marker of istiocitic cells; *PTH-receptor* parathormone receptor, marker of osteoclastic cells)

PCNA	Fibronectine	CD68	PTH-receptor
+	+	–	–
+	–	+	–
–	–	+	+

Table 3 Immunophenotype of multinuclear giant cells

PCNA	Fibronectine	CD68	PTH-receptor
–	–	+	+

cells and very small numbers of giant cells. The blood-injected joints showed brown pigmentation of synovial membrane, foam cells, oedema and congestion, but did not show villi or nodules. The plasma and gum acacia did not produce significant changes. Saline solution or blood injection into the ankles of dogs produced a non-specific synovitis [17]. Continuous hemarthrosis in the knees of dogs caused brown-staining of the synovium within 5–10 weeks and synovial hypertrophy 12–16 weeks later [6]. Round cell infiltration, hemosiderin and synovial hyperplasia were present, but no giant cells were found. These results indicated that although many features of PVNS are reproducible, the disease cannot be induced in animal models.

If trauma and inflammatory reaction may explain some aspects of PVNS, much experimental evidence supports a neoplastic aetiology. Some authors claim that the insignificant degree of inflammation, the nodular growth pattern, the propensity for recurrence after inadequate removal and the lack of characteristic changes in the adjacent synovial tissue suggest that PVNS is not an inflammatory synovitis. Moreover they argue that there does not seem to be a relationship between the degree of fibrosis and either the duration of symptoms or the size of the lesion, and no histological evidence of progressive fibrotic changes was seen [25].

Cases of malignant PVNS have been reported [3, 5]. Some authors have described the presence of cytogenetic clonal aberrations in short-term cultures and in uncultured PVNS cells, by using a fluorescence in situ hybridization technique [10, 18, 26]. The finding of acquired clonal chromosome aberrations has usually been regarded as evidence that the lesion is a true neoplasm, although clonal chromosomal changes are also present in non-neoplastic cells. The most frequent chromosome changes, among the ten cases reported in the literature, is an increase of chromosomes 5 or 7. Moreover, chromosome regions involved more than once were 1p10-p34 in four cases, 5q31-35 in two cases and 15q in two cases [24]. The presence of an extra chromosome 7 is correlated with an increase of proliferative activity in several tumours [14, 16].

In order to understand the aetiology, it is essential to clarify the origin of the cells involved. Electron microscopic studies have suggested that the different types of cells in PVNS may be derived from a basic undifferentiat-

ed mesenchymal cell [2, 8, 11]. However, it is more likely that the cells in PVNS are not derived from single stem cells; Sakkers et al. and Vogrincic et al. demonstrated, by assaying clonality in tissue from 46 XX subjects based on preferential X-chromosome inactivation analysis, that the cells in PVNS are not derived from a single stem cell [28, 31]. The co-presence of cells with cytogenetic normal metaphases, commonly observed, may reflect infiltrative non-neoplastic cells in the PVNS specimen. It may be that PVNS is a neoplastic lesion where tumour cells coexist with monocyte-macrophage cells. The antigenic pattern on immunohistochemical analysis is characterised by the presence of cells with different immunophenotypes: either fibroblastic or monocytic-macrophagic [22] (Tables 2, 3).

It is important to emphasise the fact that multinuclear giant cells are PCNA negative, i.e. not in proliferation. Studies have shown that giant cells in PVNS are formed by fusion of mononucleated precursors and express an osteoclast phenotype [7, 23, 33]. Osteoclasts may be derived from blood monocytes.

If a neoplastic aetiology is proposed, one must assume that there is a neoplastic fibroblastic-like cellular population that may produce chemotactic factors for blood monocytes which may migrate into the lesion. A similar mechanism has been recently demonstrated in another tumour [34] and the abundant production of monocyte chemo-attractant protein 1, which is a chemotactic factor specific for monocytes, has been demonstrated in cultures of giant cell tumour of bone, which is a tumour with histological, ultrastructural and immunohistochemical characteristics similar to PVNS.

In our study we only chose specimens from the localised form of PVNS because the size of the nodules, which is proportional to the duration of the disease and to the rapidity of growth, is measurable more easily than in the diffuse form. Nodular lesions can also be assumed as spheres where our measurements represent their diameters. Accordingly, monodimensional measurements can be regarded as an index of the volume of the lesion as well as an index of the duration of the disease. The point that we intended to highlight in this study is that the proliferalitive activity increases with the duration of the disease. Our data showed that PCNA-LI correlates with the dimension of nodules in PVNS. It is well known that genetic mutations may develop and accumulate with successive mitoses. The proliferative cellular activity may become more pronounced during this process. Our data suggest that the lesion may be caused by an increase in cellular proliferative activity as observed in models of neoplastic progression. A sequential increase in the PCNA-LI was observed during neoplastic progression of lesions of the colon [29] and the PCNA-LI was higher in oesophageal carcinoma than dysplasia [20].

On the basis of the evidence and our experience we believe that PVNS is a neoplastic condition and surgical treatment should be performed accordingly. Particular care is required in the presence of bony erosions and complete excision with curettage of the cavities is required to avoid recurrence.

References

1. Agarwal S, Jain R, Rusia U, Gupta RL (1997) Proliferating cell nuclear antigen immunostaining in breast carcinoma and its relationship to clinical and pathological variables. Indian J Pathol Microbiol 40:11–16
2. Alguacil GA, Unni KK, Goellner JR (1978) Giant cell tumor of tendon sheath and pigmented villonodular synovitis. An ultrastructural study. Am J Clin Pathol 69:6–17
3. Bertoni F, Unni KK, Beabout JW, Sim FH (1997) Malignant giant cell tumor of the tendon sheaths and joints (malignant pigmented villonodular synovitis). Am J Surg Pathol 21: 153–163
4. Bravo R, Frank R, Blundell PA, Macdonald Bravo H (1987) Cyclin/PCNA is the auxiliary protein of DNA polymerase-delta. Nature 326:515–517
5. Choong PFM, Willen H, Nilbert M, Mertens F, Mandahl N, Carlén B, Rydholm A (1995) Pigmented villonodular synovitis. Monoclonality and metastasis – a case for neoplastic origin? Acta Orthop Scand 66:64–68
6. Convery FR, Woo SL, Akeson WH, Amiel D, Malcolm LL (1976) Experimental hemarthrosis in the knee of the mature canine. Arthritis Rheum 19:59–67
7. Darling JM, Goldring SR, Harada Y, Handel ML, Glowacki J, Gravallese EM (1997) Multinucleated cells in pigmented villonodular synovitis and giant cell tumor of tendon sheath express features of osteoclasts. Am J Pathol 150:1383–1393
8. Eisenstein R (1968) Giant-cell tumor of tendon sheath. Its histogenesis as studied in the electron microscope. J Bone Joint Surg [Am] 50:476–486
9. Enzinger FM, Weiss SW (1995) Soft tissue tumors, 3rd edn. Mosby, St. Louis
10. Fletcher JA, Henkle C, Atkins L, Rosenberg AE, Morton CC (1992) Trisomy 5 and trisomy 7 are non random aberrations in pigmented villonodular synovitis: confirmation of trisomy 7 in uncultured cells. Genes Chromosomes Cancer 4:264–266
11. Hirohata K (1968) Light microscopic and electron microscopic studies of individual cells in pigmented villonodular synovitis and bursitis. Kobe J Med Sci 14:251–279
12. Jaffe HL, Lichtenstein L, Sutro CJ (1941) Pigmented villonodular synovitis, bursitis, and tenosynovitis. Arch Pathol 31: 731–765
13. Jaskulski D, De Riel JK, Mercer WE, Calabretta B, Baserga R (1988) Inhibition of cellular proliferation by antisense oligodeoxynucleotides to PCNA cyclin. Science 240:1544–1546
14. Lavezzi A, Mantovani M, Cazzulo A, Turconi P, Matturri L (1998) Significance of trisomy 7 related to PCNA index in cholesteatoma. Am J Otolaryngol 19:109–112
15. Mathews MB, Bernstein RM, Franza BR Jr, Garrels JI (1984) Identity of the proliferating cell nuclear antigen and cyclin. Nature 309:374–376
16. Matturri L, Biondo B, Cazzullo A, Montanari E, Radice F, Timossi R, Turconi P, Lavezzi AM (1998) Detection of trisomy 7 with fluorescence in situ hybridization and its correlation with DNA content and proliferating cell nuclear antigen-positivity in prostate cancer. Am J Clin Oncol 21:253–257
17. McCollum DE, Musser AW, Rhangos WC (1966) Experimental villonodular synovitis. South Med J 59:966–970
18. Mertens F, Orndal C, Mandahl N, Heim S, Bauer HF, Rydholm A, Tufvesson A, Willen H, Mitelman F (1993) Chromosome aberrations in tenosynovial giant cell tumors and nontumorous synovial tissue. Genes Chromosomes Cancer 6: 212–217
19. Morris G, Mathews MB (1989) Regulation of proliferating cell nuclear antigen during the cell cycle. J Biol Chem 264: 13856–13864
20. Murakami S, Uchida Y, Takeno S, Noguchi T, Matsumoto K, Shimoda H (1997) Expression of PCNA and p53 in esophageal dysplasia and esophageal carcinoma. Surg Today 27:593–599
21. Myers BW, Masi AT (1980) Pigmented villonodular synovitis and tenosynovitis: a clinical epidemiologic study of 166 cases and literature review. Medicine 59:223–238

22. Nakashima M, Ito M, Ohtsuru A, Alipov GK, Matsuzaki S, Nakayama T, Yamashita S, Sekine I (1996) Expression of parathyroid hormone (PTH)-related peptide (PTHrP) and PTH/PTHrP receptor in giant cell tumour of tendon sheath. J Pathol 180:80–84
23. Neale SD, Kristelly R, Gundle R, Quinn JM, Athanasou NA (1997) Giant cells in pigmented villo-nodular synovitis express an osteoclast phenotype. J Clin Pathol 50:605–608
24. Ohjimi Y, Iwasaki H, Ishiguro M, Kaneko Y, Tashiro H, Emoto G, Ogata K, Kikuchi M (1996) Short arm of chromosome 1 aberration recurrently found in pigmented villonodular synovitis. Cancer Genet Cytogenet 90: 80–85
25. Rao AS, Vigorita VJ (1984) Pigmented villonodular synovitis (giant-cell tumor of the tendon sheath and synovial membrane). A review of eighty-one cases. J Bone Joint Surg [Am] 66:76–94
26. Ray RA, Morton CC, Lipinski KK, Corson JM, Fletcher JA (1991) Cytogenetic evidence of clonality in a case of pigmented villonodular synovitis. Cancer 67:121–125
27. Robbins BA, De la Vega D, Ogata K, Tan EM, Nakamura RM (1987) Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignancies. Arch Pathol Lab Med 111:841–845
28. Sakkers RJB, De Jong D, Van Der Heul RO (1991) X-Chromosome inactivation in patients who have pigmented villonodular synovitis. J Bone Joint Surg [Am] 10:1532–1536
29. Shpitz B, Bomstein Y, Mekori Y, Cohen R, Kaufman Z, Grankin M, Bernheim J (1997) Proliferating cell nuclear antigen as a marker of cell kinetics in aberrant crypt foci, hyperplastic polyps, adenomas, and adenocarcinomas of the human colon. Am J Surg 174:425–430
30. Singh R, Grewal DS, Chakravarti RN (1969) Experimental production of pigmented villonodular synovitis in the knee and ankle joints of Rhesus monkeys. J Pathol 98:137–142
31. Vogrincic GS, O'Connell JX, Gilks CB (1997) Giant cell tumor of tendon sheath is a polyclonal cellular proliferation. Hum Pathol 28:815–819
32. Volz RG, Peltier LF (1963) Experimental production of pigmented villonodular synovitis in rabbits. Surg Forum 14:452
33. Wood GS, Beckstead JH, Medeiros LJ, Kempson RL, Warnke RA (1988) The cells of giant cell tumor of tendon sheath resemble osteoclasts. Am J Surg Pathol 12:444–452
34. Zheng MH, Fan Y, Smith A, Wysocki S, Papadimitriou JM, Wood DJ (1998) Gene expression of monocyte chemoattractant protein-1 in giant cell tumors of bone osteoclastoma: possible involvement in CD68+ macrophage-like cell migration. J Cell Biochem 70:121–129