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CELL PROLIFERATION STUDIES IN PRIMARY SYNOVIAL CHONDROMATOSIS

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SUMMARY

Primary synovial chondromatosis (PSC) is thought to be a cartilaginous metaplasia, but it may recur locally and malignant change has been reported. Histologically, the cartilage is usually cellular, with binucleate forms. These findings suggest that the disease is not simply a metaplasia but imply a proliferative component. In this study, immunohistochemical detection of Ki-67 protein using an antigen retrieval microwave heating technique and DNA image cytometry (VIDAS image analysis system) has been used to assess the proliferative activity in 20 cases of PSC and the results have been compared with those obtained in other cartilage tissues: ten enchondromas, ten chondrosarcomas, and ten samples of normal articular cartilage. There was no detectable staining for Ki-67 protein in cases of PSC or in benign tissues, but there was a significant association between Ki-67 labelling index and grade in the chondrosarcomas ($P<0{\cdot}01$). The absence of mitotic figures and the lack of Ki-67 protein in PSC are consistent with a metaplasia. All enchondromas gave diploid DNA histograms but non-diploid histograms were obtained in eight cases (40 per cent) of PSC, with significant populations of hyperdiploid and DNA aneuploid cells. The mean DNA content, the percentage of hyperdiploid cells, the percentage of DNA aneuploid cells, and the 2c deviation index were all significantly higher in PSC than in enchondromas ($P<0{\cdot}01$). These findings with image cytometry suggest a proliferative process in the development of at least some cases of PSC. In terms of cell proliferative activity, PSC appears to occupy a position which is intermediate between benign enchondromas and malignant chondrosarcomas, which may explain the aggressive clinical behaviour occasionally seen in this condition. © **1998 John Wiley & Sons, Ltd.**

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KEY WORDS—**synovial chondromatosis; Ki-67 protein; DNA aneuploidy; image analysis**

INTRODUCTION

Primary synovial chondromatosis (PSC) is a rare condition characterized by the formation of multiple cartilaginous nodules in the synovium of joints, tendons, and bursae.[1–3] It is seen twice as commonly in men as in women; the average age is 40 years; and the presenting symptoms are pain, swelling, and loss of joint movement. The condition is almost always monarticular, the knee being by far the most common site, accounting for two-thirds of all cases. It occurs nearly always within joints, but extra-articular disease has been described.[4–6]

The cause of PSC is not known. There is no familial history, no occupational history, and usually no convincing history of previous trauma.[1] It is thought to be a cartilaginous metaplasia,[7,8] but the cartilage is often very cellular, with binucleate forms and marked nuclear pleomorphism which would be interpreted as malignant if it occurred within a bone. Furthermore, it may recur locally and malignant change has been reported.[9–12] These findings suggest that the condition is not solely a metaplasia, but has a proliferative component in at least some cases.

In this study, immunohistochemical techniques (Ki-67) and DNA image cytometry have been used to assess the proliferative status of the cartilage in PSC and the results have been compared with normal articular cartilage, enchondromas, and chondrosarcomas in an attempt to determine if there is significant proliferation of the cartilage in this condition.

MATERIALS AND METHODS

Twenty cases of synovial chondromatosis have been examined along with ten enchondromas, ten chondrosarcomas, and ten samples of normal articular cartilage obtained from femoral heads removed for avascular necrosis. In addition, a recent case of synovial chondromatosis which underwent malignant transformation was also available.

Histological studies

New 4 μm sections were cut and visually assessed for cellularity of cartilage, the presence of pleomorphic cells, binucleate cells, mitotic figures, and the extent of calcification and ossification. Three-point scales were used to grade the degree of cellularity (1 = mild; 2 = moderate; 3 = markedly increased), nuclear pleomorphism (1 = little; 2 = moderate; 3 = marked variation in cell nuclei), and the extent of calcification/ossification within the cartilage (1 = less than 10 per cent; 2 = 10–50 per cent; 3 = more

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than 50 per cent). Chondrosarcomas were graded using the criteria of Evans *et al.*[13]

Immunohistochemical studies

Sections 4 μm thick were cut from wax blocks and placed on APES-coated slides. After dewaxing in xylene, endogenous peroxidase activity was blocked with 1·5 per cent hydrogen peroxide in methanol for 10 min.

As all cases were from paraffin-embedded tissue, an antigen retrieval method similar to that described by Shi *et al.*[14] was used. Sections were immersed in 10 mM citrate buffer (pH 6·0) and microwave-treated for 20 min (predetermined by control studies) at full power using a Toshiba 750W domestic model, checking for evaporation at 5-min intervals and topping up as required. The sections were then left to stand in this buffer for 15–20 min. For the immunostaining, polyclonal Ki-67 antibody (DAKO) was used at 1 in 100 dilution.

Antibody was applied for 30 min at 37°C followed by a streptavidin biotin procedure using a DAKO duet kit (DAKO K492), colour development with 0·05 per cent diaminobenzidine in hydrogen peroxide, and counterstaining with haematoxylin.

A positive control section of tonsil was included in each immunostaining run. Negative controls involved following the same procedure with omission of the primary antibody. As all cases had been subjected to varying degrees of decalcification in acid, a series of control experiments was performed to determine the effect of prolonged exposure to formalin and formic acid on Ki-67 immunostaining. Slices of normal tonsil were placed in 10 per cent buffered formalin followed by 5 per cent formic acid for varying times (24–72 h) and then processed and embedded. The above procedure for antigen retrieval and immunostaining was followed and the staining extent and intensity were assessed.

Chondrocytes with sharp staining of nuclei (irrespective of intensity) and clear cytoplasm were designated as Ki-67-positive. The labelling index was determined by counting the number of Ki-67-positive cells amongst 1000 cells from randomly selected areas at $\times 400$ magnification.

Image cytometry

Image cytometry was performed on Feulgen-stained tissue sections using a VIDAS image analysis section (Kontron, Germany). As the normal articular cartilage was of such low cellularity, DNA densitometry was not performed in these cases. Digitally captured images were stored on optical disc, analysed with a computer, and DNA histograms were obtained. A modification of the method described by Kreicbergs and Zetterberg[15] was followed.

Sections 4 μm thick were cut from wax blocks and deparaffinized in xylene. Duplicate sections were placed in 1 M hydrochloric acid (HCl) at 60°C, removing one section after 10 min and the other after 20 min. The sections were then placed in Schiff's reagent for 15 min, followed by three 1-min rinses in 0·5 per cent sodium bisulphite.

Control cells (lymphocytes and fibroblasts) and chondrocytes could easily be distinguished on these Feulgen-stained sections by comparison with the corresponding haematoxylin and eosin-stained sections.

Sections were examined under an oil immersion lens (100:1) using an Olympus BH2 microscope and images of nuclei were captured using a Sony single-chip CCD high-resolution CCD camera. In each case, 50 control cells and 100 chondrocyte cells were analysed. Cells were selected from different fields so as to obtain as representative a sample as possible. In every case, a DNA histogram was plotted by computer. The histograms were classified by shape into four types, a modification of Auer *et al.*:[16]

Type IA— a single peak around the 2c value with a standard deviation of $\leq 0{\cdot}5$c.
Type IB— a single peak around the 2c value with a standard deviation of $>0{\cdot}5$c.
Type II— a distinct modal value in the tetraploid (4c) region, or two distinct peaks around the diploid and tetraploid regions.
Type III— two peaks with a 5c exceeding rate of <10 per cent.
Type IV— any histogram with a 5c exceeding rate of ≥ 10 per cent.

The computer also calculated the mean C level, the standard deviation (SD), the 2·5c exceeding rate (percentage hyperdiploid cells), the 5c exceeding rate (percentage aneuploid cells), and the 2c deviation index.

Statistics

Data were collected and analysed using the Statistical Package for Social Sciences (SPSS) software. Mean C, 2·5cER, 5cER, and 2cDI for cases of synovial chondromatosis, enchondromas, and chondrosarcomas were compared using the Kruskal–Wallis one-way analysis of variance. *P* values of less than 0·05 were accepted as being significant.

RESULTS

In cases of synovial chondromatosis, there were 12 males and eight females, with a mean age of 39 ± 8 years (range 18–50 years). Thirteen cases occurred in the knee; four in the hip joint; and one each in the elbow, finger, and foot. The histological features are summarized in Table I. Binucleate chondrocytes were easily identified in all cases, but mitotic figures were not identified in any cases. The case of synovial chondromatosis which became malignant occurred in the knee of a 44-year-old man.

No mitotic figures were seen in any of the enchondromas, although binucleate cells were present in all cases. The ten chondrosarcomas were of the following grade: two grade I, five grade II, two grade III, and one dedifferentiated.

The normal articular cartilage was typically poorly cellular in all cases, especially in comparison with the

Table I—Histological features in synovial chondromatosis ($n=20$)

Feature	Grade 0	1	2	3
Cellularity	0	3	11	6
Pleomorphism	0	12	8	0
Calcification extent	7	10	2	1
Ossification extent	11	7	1	1

other cases. Cellular pleomorphism was not a feature in any case; binucleate cells were very sparse and mitotic figures were absent.

Immunohistochemical studies

There was strong nuclear Ki-67 staining in sections of normal tonsil. There was a slight reduction in staining intensity but no significant loss of staining extent when tissue was subjected to 24 h treatment in acid after 24 h in formalin fixative. There was no reduction in staining extent or intensity with acid treatment if tissues were fixed for more than 24 h in formalin.

Table II—Classification of histograms (modified Auer's)

Group	Type IA	IB	II	III	IV
Synovial chondromatosis ($n=20$)	3	9	—	4	4
Enchondroma ($n=10$)	2	8	—	—	—
Chondrosarcoma ($n=11$)	1	4	—	3	3

There was no identifiable Ki-67 staining in the chondrocytes in any of the cases of synovial chondromatosis, enchondroma, or normal articular cartilage. At least 1000 chondrocytes were observed and assessed in all but four cases, where 500 cells were assessed. In the chondrosarcomas, the Ki-67 labelling index ranged from 0·4 per cent in a grade I tumour to 11 per cent in the dedifferentiated tumour. The mean Ki-67 index was $3{\cdot}6 \pm 3{\cdot}5$ per cent. There was a significant association between Ki-67 index and grade of tumour ($P<0{\cdot}01$).

In the case of synovial chondromatosis which became malignant, no Ki-67 staining was detected in the original biopsy showing the features of synovial chondromatosis. In sections taken from the subsequent chondrosarcoma, there was a Ki-67 proliferation index of 4 per cent.

Image cytometry

All cases had been decalcified in formic acid, but a satisfactory Feulgen stain was obtained with well-stained nuclei in a clean unstained background.

When the histograms were analysed, there was complete agreement between the observers (RID and PWH). The results are summarized in Table II. All the enchondromas had diploid histograms, but diploid histograms were obtained in only 12 of the 20 cases of synovial chondromatosis and five of 11 chondrosarcomas (Figs 1–4).

DNA densitometry variables are summarized in Table III. There was a trend for increasing mean C value, but this was not significant ($P=0{\cdot}2$). The mean 2·5cER ($P=0{\cdot}04$), 5cER ($P=0{\cdot}007$), and 2cDI ($P=0{\cdot}002$) were significantly greater in synovial chondromatosis and chondrosarcomas than in the enchondromas.

DISCUSSION

In order to determine whether this condition is a metaplasia or hyperplasia, it would ideally be desirable

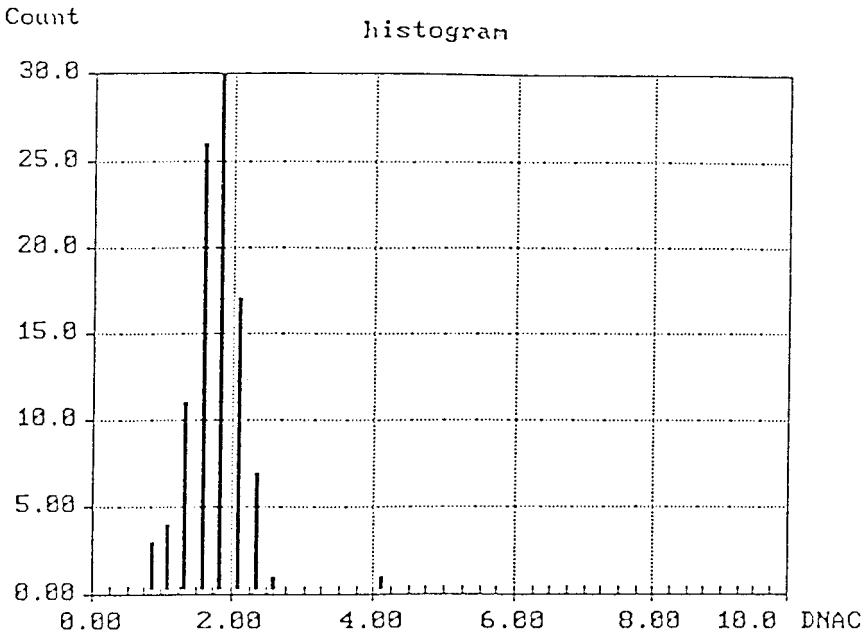

Fig. 1—DNA diploid histogram in an enchondroma

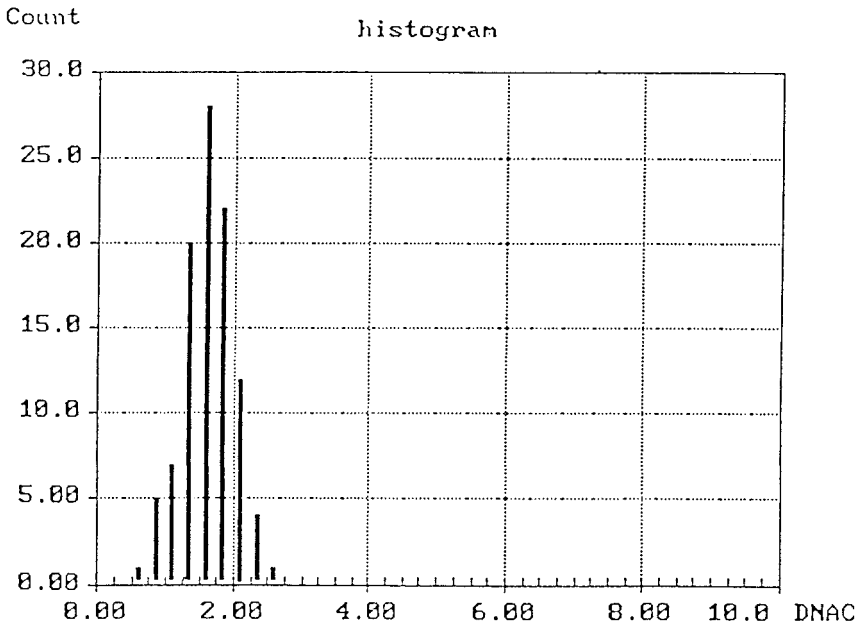

Fig. 2—DNA diploid histogram in synovial chondromatosis

to compare cases of synovial chondromatosis not only with other cartilaginous neoplasms, but also with examples of chondroid metaplasia and hyperplasia. Since true chondroid metaplasias and hyperplasias are rare and are not routinely biopsied, this was not practicable.

The cartilage in synovial chondromatosis was of increased cellularity in all cases and was at least moderately cellular in 17 of the 20 cases. There was also significant nuclear pleomorphism of the chondrocytes. The histological appearances were indistinguishable from the low-grade chondrosarcomas. In contrast, the normal articular cartilage was relatively poor in cellularity.

Mitotic figures were identified in the chondrosarcomas but not in cases of synovial chondromatosis or in the enchondromas. However, binucleate chondrocytes were present in all cases of synovial chondromatosis. Counting mitotic figures is a well-established and elementary method of assessing cell proliferation in tumours,[17,18] although the technique has limitations, due to lack of reproducibility and fixation effects.[19,20] The lack of mitoses does not suggest a lesion with active cell proliferation.

Ki-67 immunohistochemical staining can be used to assess the proliferative activity in normal, reactive, and neoplastic tissues.[21–23] There was no detectable staining

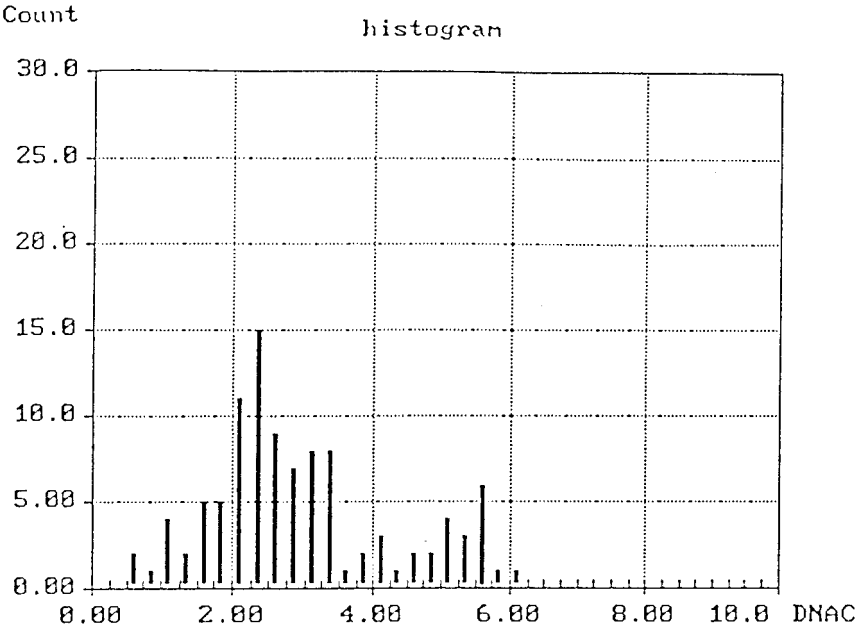

Fig. 3—Type IV DNA histogram in synovial chondromatosis

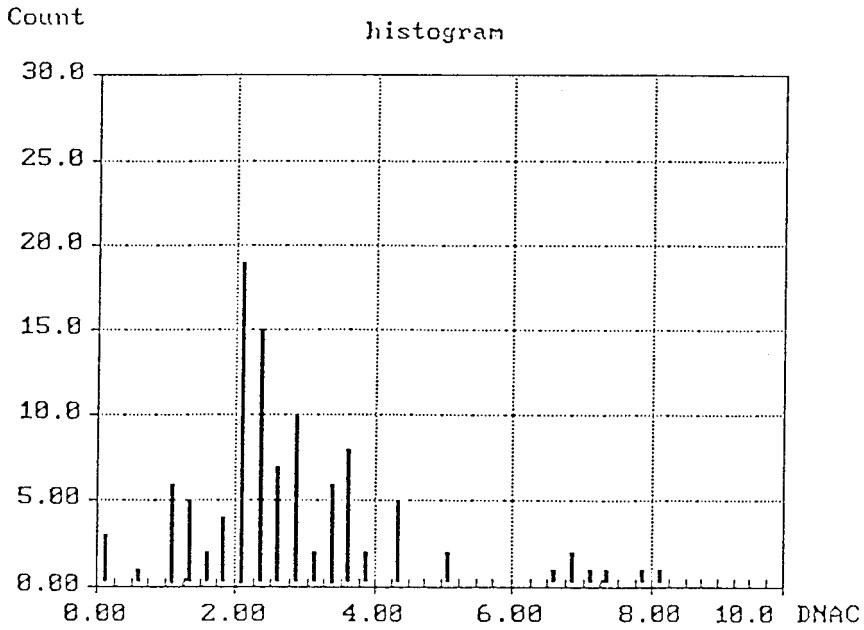

Fig. 4—Type IV DNA histogram in chondrosarcoma

for Ki-67 protein in any of the cases of synovial chondromatosis or enchondromas. A strong association was observed between Ki-67 proliferation index and grade in the chondrosarcomas ($P<0{\cdot}01$), in agreement with previous studies.[24,25] The lack of Ki-67 staining in synovial chondromatosis is in keeping with the findings of others,[8] who found that PSC did not express this antigen and concluded that the cartilage nodules were formed by a metaplastic process. The negative immunostaining with Ki-67 in this study is unlikely to be due to the effects of overfixation or the effects of acid hydrolysis, as the control studies with tonsil showed no loss of staining extent when tissue was subjected to acid treatment or prolonged formalin fixation.

We have previously reported results for C-erb B-2 staining in these cartilaginous lesions.[26] Cytoplasmic immunostaining for C-erb B-2 protein was noted in 15 of 20 cases of synovial chondromatosis, but was never seen in normal articular cartilage, enchondromas, or grade I chondrosarcomas. This is in agreement with Wrba *et al.*,[27] who reported similar findings in a study of cartilaginous neoplasms and normal articular cartilage, although their study did not include cases of PSC. In view of the negative Ki-67 immunostaining in these cases of synovial chondromatosis, the explanation of the C-erb B-2 staining is unclear. It has been suggested that cytoplasmic staining for C-erb B-2 may represent an intermediate protein product.[28–30] Alternatively, detection of C-erb B-2 protein may be a more sensitive indicator of cell proliferation than nuclear expression of Ki-67 protein.

Image cytometry has demonstrated clear diploid histograms (type 1) in all the enchondromas and grade I chondrosarcomas, in agreement with others.[31–34] Eight cases of synovial chondromatosis (40 per cent) were not diploid, with significant populations of hyperdiploid and DNA aneuploid cells. Matsuno *et al.*[35] found no DNA aneuploid cells in two cases of PSC. Coughlan *et al.*[34] looked at six cases but all were diploid. The significance of DNA aneuploid cells in benign lesions is uncertain. Hyperplastic tissues may mimic DNA aneuploidy[36] and small numbers of tetraploid nuclei have been found in chondromas.[31] There was marked hypercellularity (grade III) and nuclear pleomorphism (grade III) in the corresponding histological sections in some of these DNA aneuploid cases. Furthermore, the mean C, 2·5cER, 5cER, and 2cDI were all significantly higher in synovial chondromatosis than in enchondromas and only slightly less than in the chondrosarcomas. These findings with image analysis suggest that in at least some cases of synovial chondromatosis, even though no Ki-67-positive cells were detected, there is quite significant cellular proliferation within the cartilage, and

Table III—DNA densitometry measurements

Group	Mean C	2·5cER	5cER	2cDI
Synovial chondromatosis ($n=20$)	$2{\cdot}4 \pm 0{\cdot}7$	$25{\cdot}6 \pm 21{\cdot}4$	5 ± 8	$1{\cdot}2 \pm 0{\cdot}7$
Enchondroma ($n=10$)	$2{\cdot}1 \pm 0{\cdot}2$	$9{\cdot}2 \pm 7{\cdot}3$	0	$0{\cdot}6 \pm 0{\cdot}1$
Chondrosarcoma ($n=11$)	$2{\cdot}6 \pm 1$	$30{\cdot}0 \pm 26{\cdot}2$	$8{\cdot}2 \pm 9$	$1{\cdot}6 \pm 1{\cdot}1$

Values are mean ± SD.

favour a proliferative origin as opposed to a simple metaplasia.

In conclusion, these findings cannot determine whether a given individual case of PSC is *either* a metaplasia *or* a proliferative process. The complete absence of mitotic figures and the lack of Ki-67 immunostaining do not indicate a proliferative process and suggest a metaplastic origin. Conversely, the results for C-erb B-2 protein expression and DNA image cytometry suggest a significant degree of cellular proliferation, at least in some cases, and would not be consistent with metaplasia alone. In terms of cellular proliferative activity (especially DNA cytometry data), synovial chondromatosis appears to occupy an intermediate position between enchondroma and chondrosarcoma. The lesion may well originate as a simple metaplasia and behave as a benign neoplasm. Clearly, however, in a significant number of cases, there is active cellular proliferation of the cartilage, which may explain the tendency of this condition to recur and its propensity for malignant transformation.

REFERENCES

1. Murphy FP, Dahlin DC, Sullivan CR. Articular synovial chondromatosis. *J Bone Jt Surg* 1962: 44A: 77–86.
2. Milgram JW. Synovial osteochondromatosis. A histopathological study of thirty cases. *J Bone Jt Surg* 1977; **59A:** 792–801.
3. Davis RI, Hayes D. Primary synovial chondromatosis; a clinicopathologic review of 32 cases. *J Pathol* 1989; **177A**.
4. Dahlin DC, Salvador AH. Cartilaginous tumours of the soft tissues of the hands and feet. *Mayo Clin Proc* 1974; **49:** 721–726.
5. Sim FH, Dahlin DC, Ivins JC. Extra-articular synovial chondromatosis. *J Bone Jt Surg* 1977; **59A:** 492–495.
6. Sviland L, Malcolm AJ. Synovial chondromatosis presenting as a painless soft tissue mass—report of nineteen cases. *Histopathology* 1995; **27:** 275–279.
7. Villacin AB, Brigham LN, Bullough PG. Primary and secondary synovial chondrometaplasia. Histopathologic and clinico-radiologic differences. *Hum Pathol* 1979; **4:** 439–451.
8. Apte S, Athanasou NA. An immunohistological study of cartilage and synovium in primary synovial chondromatosis. *J Pathol* 1992; **166:** 277–281.
9. Hamilton A, Davis RI, Nixon JR. Synovial chondrosarcoma complicating synovial chondromatosis. Report of a case and review of the literature. *J Bone Jt Surg* 1987; **69A:** 1084–1088.
10. Bertoni F, Unni KK, Beabout JW, Sim FH. Chondrosarcomas of the synovium. *Cancer* 1991; **67:** 155–162.
11. Kenan S, Abdelwahab IF, Klein MJ, Lewis MD. Case report 817. *Skeletal Radiol* 1993; **22:** 623–626.
12. Ontell F, Greenspan A. Chondrosarcoma complicating synovial chondromatosis: findings with magnetic resonance imaging. *Can Assoc Radiol J* 1994; **45:** 318–323.
13. Evans HL, Ayala AG, Romsdahl MM. Prognostic factors in chondrosarcoma of bone. A clinicopathologic analysis with emphasis on histologic grading. *Cancer* 1977; **40:** 818–831.
14. Shi S-R, Key ME, Kalra ML. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 1991; **39:** 741–748.
15. Kreicbergs A, Zetterberg A. Cytophotometric DNA measurements of chondrosarcoma. Methodologic aspects of measurements in tissue sections from old paraffin embedded specimens. *Anal Quant Cytol* 1980; **2:** 84–92.
16. Auer GU, Caspersson TO, Wallgren AS. DNA content and survival in mammary carcinoma. *Anal Quant Cytol* 1980; **2:** 161–165.
17. Hall PA, Levison DA. Assessment of cell proliferation in histological material. *J Clin Pathol* 1990; **43:** 184–192.
18. Quinn CM, Wright NA. The clinical assessment of proliferation and growth in human tumours: evaluation of methods and applications as prognostic variables. *J Pathol* 1990; **160:** 93–102.
19. Silverberg SG. Reproducibility of the mitosis count in the histologic diagnosis of smooth muscle tumours of the uterus. *Hum Pathol* 1976; **7:** 451–454.
20. Cross SS, Start RD, Smith JHF. Does delay in fixation affect the number of mitotic figures in processed tissue? *J Clin Pathol* 1990; **43:** 597–599.
21. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 1983; **31:** 13–20.
22. Gerdes J, Lemke H, Baisch H, Wacker H-H, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* 1984; **133:** 1710–1715.
23. Brown DC, Gatter KC. Monoclonal antibody Ki-67: its use in histopathology. *Histopathology* 1990; **17:** 489–503.
24. Vollmer E, Roessner A, Gerdes J, *et al*. Improved grading of bone tumours with the monoclonal antibody Ki-67. *J Cancer Res Clin Oncol* 1986; **112:** 281–282.
25. Scotlandi K, Serra M, Manara MC, *et al*. Clinical relevance of Ki-67 expression in bone tumours. *Cancer* 1995; **75:** 806–814.
26. Davis RI, Foster H, Biggart JD. C-erb B-2 staining in primary synovial chondromatosis: a comparison with other cartilaginous tumours. *J Pathol* 1996; **179:** 392–395.
27. Wrba F, Gullick WJ, Fertl H, Amann G, Salzer-Kuntschik M. Immunohistochemical detection of the C-erb B-2 proto-oncogene product in normal, benign and malignant cartilage tissues. *Histopathology* 1989; **15:** 71–76.
28. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of human C-erb B-2 gene: a 185 kilodalton glycoprotein with tyrosine kinase activity. *Science* 1986; **232:** 1644–1646.
29. De Potter CR, Van Daele S, Van de Vijver MJ, *et al*. The expression of the neu oncogene product in breast lesions and in normal fetal and adult human tissues. *Histopathology* 1989; **15:** 351–362.
30. Lofts FJ, Gullick WJ. C-erb B-2 amplification and overexpression in human tumours. *Can Treat Res* 1992; **61:** 161–164.
31. Cuvelier C, Roels HJ. Cytophotometric studies of the nuclear DNA content in cartilaginous tumours. *Cancer* 1979; **44:** 1363–1374.
32. Kreicbergs A, Zetterberg A, Soderberg G. The prognostic significance of nuclear DNA content in chondrosarcoma. *Anal Quant Cytol* 1980; **2:** 272–279.
33. Kreicbergs A, Boquist L, Borssen B, Larsson SE. Prognostic factors in chondrosarcoma. A comparative study of cellular DNA content and clinicopathologic features. *Cancer* 1982; **50:** 577–583.
34. Coughlan B, Feliz A, Ishida T, Czerniak B, Dorfman HD. p53 expression and DNA ploidy of cartilage lesions. *Hum Pathol* 1995; **26:** 620–624.
35. Matsuno T, Gebhardt MC, Schiller AL, Rosenberg AE, Mankin HJ. The use of flow cytometry as a diagnostic aid in the management of soft tissue tumours. *J Bone Jt Surg* 1988; **70A:** 751–759.
36. Stephenson TJ. Quantitation of the nucleus. In: Williams GT, ed. Current Topics in Pathology 81. Gastrointestinal Pathology. Berlin: Springer-Verlag, 1990; 182.