Acta Orthop. Scand. *58*, 523–528, 1987

# Aspiration of musculoskeletal tumors for cytodiagnosis and DNA analysis

**Måns Åkerman, Dick Killander[1], Anders Rydholm[2] and Bo Rööser[2]**

Preoperative fine-needle aspirates of 25 soft tissue tumors and four bone tumors were used for cytodiagnosis and flow cytometric DNA ploidy analysis. The results were compared with the histopathologic diagnosis and flow cytometric DNA ploidy analysis on tissue samples after surgery.

There was good agreement between the cytodiagnoses and histopathologic diagnoses with regard to benign tumor or sarcoma. There was good agreement between the ploidy determinations in aspirates and tissue samples in 20 out of the 26 tumors that could be analyzed. Aneuploid cell populations were found in 13 tumors cytologically classified as sarcoma and in one tumor classified as unspecified malignant tumor. These 14 tumors also showed aneuploid cell populations in the tissue samples and were histologically diagnosed as high-grade malignant sarcomas.

The combined evaluation of preoperative cytodiagnosis and DNA ploidy on aspirates may give valuable prognostic information.

The prognostic value of DNA flow cytometry is well established in several oncologic fields, but there are only a few reports on this technique in musculoskeletal tumors (Kreicbergs et al. 1980, 1982, Keen et al. 1985, Mankin et al. 1985). In chondrosarcoma the DNA ploidy level is a key factor for survival (Kreicbergs et al. 1980); guidelines for treatment emerged when the prognosis was based on a combination of DNA analysis with other factors, notably tumor size and histologic malignancy grade.

In soft tissue sarcomas, however, open biopsy should be avoided (Stener 1979, Rydholm et al. 1986); aspiration cytology (Åkerman et al. 1985) may then replace histologic studies.

We report the use of fine-needle aspirates collected preoperatively for cytologic and flow cytometric DNA analysis.

Lund University Departments of Cytodiagnostics, [1]Oncology, and [2]Orthopedics, S-221 85 Lund, Sweden

Correspondence: Dr. Måns Åkerman, Department of Cytodiagnostics, University Hospital, S-221 85, Lund, Sweden

## Patients and methods

*Patients.* Twenty-nine patients with musculoskeletal tumors, referred to us before treatment, were analyzed during the period August 1984-March 1986. Four patients had bone tumors; one a giant cell tumor, one a chondrosarcoma, one Ewing's sarcoma, and one an osteosarcoma (Table 1). Two out of the 25 soft tissue tumors were benign, three were low-grade malignant (Grades I and II), and 20 were high-grade malignant (Grades III or IV) on a four-grade scale (Markhede et al. 1982, Rydholm et al. 1984).

*Cytology.* Fine-needle aspiration was performed preoperatively (Zajicek 1974). The immediate cytologic report was either benign or sarcoma, unspecified malignant tumor, inconclusive (with regard to benignity or malignancy), or insufficient material for diagnosis. The 26 sarcomas were also classified as low- or high-grade malignant in 16, and a histogenetic type was suggested in 14 (Table 1).

*DNA analysis.* Cells for flow cytometric DNA analysis were obtained after aspiration and smearing by flushing the needle and syringe with ice cold 96 per cent ethanol.

Macroscopically non-necrotic, fresh tumor tissue from 1–3 different sites were taken immediately after surgery. The tissue was snap frozen in isopenthane and stored at -70° C until analysis. The preparation and staining methods were developed (Ewers et al. 1984) as modifications of the method of Vindelov (1977) and Tribukait et al. (1979). The tumor tissue sample was squeezed through a 140-$\mu$m nylon filter and Tris buffer was added. The cell suspension was centrifuged for 15 min at 150 G. The pellet was shaken intensely in a supermixer with 2 ml of absolute ethanol cooled to −20° C and stored in 4° C until further processing. After a new centrifugation the cellular pellet obtained from tissue or from aspirates was resuspended in 1 ml of RNA-ase solution (bovine pancreas RNA-ase A type III-A, Sigma) and left for 5 min at room temperature. After pelleting again the cells were incubated in 1 ml of 0.5 per cent pepsin solution at 37° C for 10 min. The sample was then cooled on ice for 5 min and after pelleting suspended in 1 ml of Tris buffer and filtered through a 50-$\mu$m nylon filter. The concentrations of nuclei were adjusted to approximately $10^6$ nuclei/ml. One milliliter of the suspension of nuclei was centrifuged for 5 min at 250 G and the supernatant was decanted. One milliliter of propidium iodine solution was added, and the suspension was filtered again through the 50-$\mu$m nylon filter just before DNA measurement. Normal human female lymphocytes were treated together with the other samples and used as an external DNA standard.

The DNA analyses were performed with an Ortho cytofluorograph 50-H system (Ortho Instruments, Westwood, MD, USA) equipped with a 4 W argon-ion laser (Model 95, Lexel Corp., Palo Alto, CA, USA), a 512 multichannel analyzer, a storage oscilloscope, and a multiparameter signal processor. Peaks corresponding to both the G1/GO and to the G2 phases of the cell cycle could be identified. With an electronic gate setting, it was possible to exclude cell doublets from the main population.

In general, 10,000–15,000 cells were measured in each case. The flow cytometric analyses always started with lymphocyte measurements, giving the normal modal DNA diploid value, and these were repeated after every third sample. The DNA index (DI) of a tumor cell population was obtained as the ratio of its modal DNA value in relation to the modal DNA value of the lymphocytes that express the normal diploid DNA amount. Strictly, a diploid cell population has, by definition, DI = 1.00. Due to various sources of errors, diploid tumors exhibit a variation in DI values around this value. Therefore, in the present work the condition for defining a tumor as aneuploid was, in analogy with the recommendations by Hiddeman et al. (1984), the presence of at least two separate modal DNA values, representing different subpopulations of cells in the tumor. Normal diploid cells (and diploid tumor cells?) contribute most likely to one of these modal DNA values.

## Results

*Cytodiagnosis.* In 28/29 patients, sufficient material was obtained for cytodiagnosis – in a lipoma (Case 2) the material was insufficient. In Case 17, a malignant fibrous histocytoma, the cytodiagnosis was inconclusive. In the remaining 27 cases the cytodiagnosis was correct with regard to benign tumor, 2 cases, or malignant tumor, 25 cases (Table 1).

Sixteen sarcomas were also graded cytologically, and in 15 the grade was the same as the histologic grade (13 high-grade and two low-grade, respectively). The histogenetic type was suggested in 14 sarcomas and corresponded with the histopathologic diagnosis in 13 sarcomas.

*DNA ploidy in aspirates and tissue.* Enough material for a flow cytometric DNA analysis was obtained in 26 of 29 patients (Table 1). The three failures were two benign tumors (Cases 1 and 2) and one sarcoma (Case 6) The DNA indices obtained from aspirates were compared with those from the tissue pieces. In 15 cases only one tissue piece was analyzed: the correlation was $r$=0.85. The combined $r$ value for all 26 cases was 0.84 ($P$<0.001). Evident discrepancies between DI values for aspirates and tissues were observed in 4 patients: Cases 17–19, 28 (Figure 1). In Cases 17 and 28, these discrepancies were due to a sampling error, as the aspirate contained mainly leukocytes, macrophages, and necrotic cells, and only a few scattered tumor cells. In Case 19 the aspirate consisted of numerous malignant cells and in Case 18 there was a cell population with

Table 1. Clinical data, morphologic diagnoses, malignancy grades, DNA indices (DI), and ploidy determination of aspirates and tissues

| Case | Sex | Age | Site | Cytodiagnosis[a]; malignancy grade[b] | Histologic diagnosis[a]; malignancy grade | Aspirate DI[c] | No. of cell populations and ploidy[d] | Fresh tissue DI[c] | No. of cell populations and ploidy[d] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 17 | Lower leg | Fib | Pro fasc | Failure | – | 0.98 | 1 E |
| 2 | F | 77 | Thigh | Insuff | Alip | Failure | – | 0.94 | 1 E |
| 3 | F | 54 | Sacrum | GCT | GCT | 1.00 | 1 E | 1.03[e]<br>1.09 | 1 E |
| 4 | F | 70 | Thigh | Sa (MFS); L | MFS; I | 0.97 | 1 E | 0.98 | 1 E |
| 5 | M | 49 | Abdominal wall | Unspec sa; L | MFS; II | 1.06 | 1 E | 1.02[e]<br>1.02 | 1 E |
| 6 | F | 67 | Thigh | MFH; H | MFH; II | Failure | – | 1.61[e]<br>1.64 | 2 A |
| 7 | M | 66 | Lower leg | Unspec sa; H | MFH; III | *0.94*[f]<br>1.56 | 3 A | 0.94 | 1 E |
| 8 | M | 33 | Foot | Sa (MFS); H | MFH; III | 1.08 | 1 E | 1.06[e]<br>1.02 | 1 E |
| 9 | M | 78 | Thigh | Unspec sa | Cc; III | 1.72 | 2 A | 1.77 | 2 A |
| 10 | F | 76 | Forearm | Sarcoma (Leio?); H | Leio; III | 1.35 | 2 A | 1.39[e]<br>1.42 | 2 A |
| 11 | M | 64 | Forearm | Unspec sa | Ec; III | 1.58 | 2 A | 1.59[e]<br>1.61<br>1.67 | 2 A<br>2 A<br>2 A |
| 12 | F | 44 | Ulna | Ch; H | Ch; III | 0.64[f]<br>*1.10* | 3 A | 0.62[f]<br>*1.15* | 3 A |
| 13 | M | 54 | Inguinal region | Unspec sa | Ne; III | 1.02 | 1 E | 0.96 | 1 E |
| 14 | M | 43 | Lower leg | Lisa; H | Lisa; III | 1.00 | 1 E | 1.01[e]<br>0.99<br>0.97 | 1 E |
| 15 | M | 8 | Ileal bone | Ew?; H | Ew; IV | 1.06 | 2 A | 1.18 | 2 A |
| 16 | M | 28 | Popliteal fossa | Sy?; H | Sy; IV | 1.00 | 1 E | 0.87 | 2 A |
| 17 | F | 77 | Inguinal region | Incon | MFH; IV | 1.05 | 1 E | 1.64 | 2 A |
| 18 | F | 53 | Scapula | Unspec mal | Os; IV | 1.56 | 2 A | 1.84[e+f]<br>*3.09*<br>1.84<br>*3.11* | 3 A |
| 19 | M | 74 | Upper arm | MFH; H | MFH; IV | 1.08 | 1 E | *1.46*[d]<br>2.85 | 3 A |
| 20 | F | 86 | Thigh | MFH; H | MFH; IV | 2.23 | 2 A | 2.33 | 2 A |
| 21 | M | 52 | Upper arm | Lisa?; H | Lisa; IV | 1.32 | 2 A | 1.25 | 2 A |
| 22 | M | 39 | Forearm | Unspec sa | Sy; IV | 1.00 | 1 E | 0.88 | 2 A |
| 23 | F | 75 | Thigh | MFH | MFH; IV | *1.89*[f]<br>3.64 | 3 A | *1.84*[e+f]<br>3.64<br>1.91<br>3.71 | 3 A<br>3 A |
| 24 | M | 67 | Thoracal region | Unspec sa | MFH; IV | 1.27[e]<br>*2.27* | 3 A | 1.44[e+f]<br>*2.46* | 3 A |
| 25 | F | 19 | Anal region | Rhab?; H | Rhab; IV | 1.76 | 2 A | 1.75 | 2 A |
| 26 | M | 59 | Back | Unspec sa; H | MFH; IV | 1.38 | 2 A | 1.35[e]<br>1.36 | 2 A<br>2 A |
| 27 | M | 61 | Knee | MFH; H | MFH; IV | 1.41 | 2 A | 1.42[e]<br>1.58 | 2 A<br>2 A |
| 28 | F | 74 | Thigh | Unspec sa | MFH; IV | 1.08 | 1 E | 0.87[f]<br>*1.74* | 3 A |
| 29 | M | 86 | Thigh | Unspec sa | MFH; IV | 1.09 | 1 E | 1.08 | 1 E |

[a] Alip atypical lipoma, Cc clear cell sarcoma, Ch chondrosarcoma, Ec Epithelioid sarcoma, Ew Ewing's sarcoma, Fib fibromatosis, GCT giant cell tumor of bone, Incon inconclusive despite sufficient material, Insuff insufficient material for diagnosis, Leio leiomyosaroma, Lisa liposarcoma, MFH malignant fibrous histiocytoma, MFS myxofibrosarcoma, Ne neurogenic sarcoma, Os osteosarcoma, Pro fasc proliferative fasciitis, Rhab rhabdomyosarcoma, Sa sarcoma, Unspec mal unspecified malignancy, Unspec sa unspecified sarcoma.

[b] L malignancy grades I, II, H malignancy grades III, IV.

[c] In cases of two or more cell populations, the DI valve of cells representing the normal diploid region is not included.

[d] E euploid, A aneuploid.

[e] Values of more than one tissue sample from different parts of the same tumor.

[f] One tumor sample with more than two cell populations with respect to DI values with the largest population in italics.

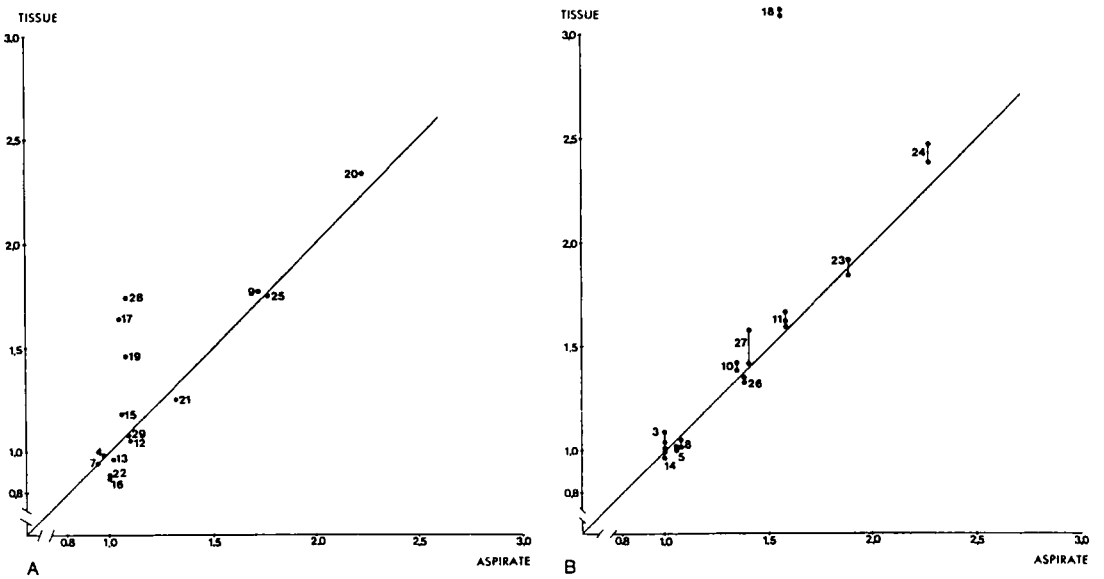


Figure 1. DNA index (DI) values obtained from aspirates and tissue. In cases with more than two cell populations with respect to DI values only the largest, in cell number, represents the case; i.e., in case of 4,000 cells in one population and 5,000 cells in the other, the DI value of the latter was chosen to represent the case.
A. Cases with only one tissue sample. $r=0.81$, $P<0.001$.
B. Cases with more than one tissue sample obtained from different parts of the same tumor where DI values are connected. $r=0.84$, $P<0.001$.

a high DI value in two different tissue samples (DI 3.09 and 3.11, respectively), which was absent in the aspirate.

The DNA values were also used for ploidy determination comparing the two cell sampling methods. The same degree of ploidy (either euploid/euploid or aneuploid/aneuploid) was obtained in 20 out of 26 patients (Table 1 and Figures 2 and 3); but in 1 (Case 18), two aneuploid cell populations were found in the tissue samples, only one in the aspirate. In the remaining 6 cases, aneuploid cell populations were found in the tissue samples, but not in the corresponding aspirates in 5 (Cases 16, 17, 19, 22, 28), and in the aspirate but not in the tissue sample in 1 (Case 7). As pointed out above, there were sampling errors in the aspirates in Cases 17 and 28; but in Cases 7, 16, 19, and 22, there were no explanations for the discrepancy in the ploidy determination.

*DNA ploidy and histologic malignancy grade.*

*Aspirates.* In only one of the three benign tumors, the aspirates could be analyzed (see above), and it was euploid. Malignancy grading and DNA ploidy determination were done in 15 out of 25 sarcoma aspirates (Table 1). Two low-grade sarcomas were euploid, four high-grade sarcomas were euploid, and nine were aneuploid.

*Tissue samples.* In three benign tumors the tissue samples were euploid. Of three histopathologically low-grade sarcomas, two were euploid and one aneuploid. Of 23 high-grade sarcomas, five were euploid (four Grade III tumors and one Grade IV tumor) and 18 aneuploid (four Grade III and 14 Grade IV tumors). Thus, with regard to sampling method – tissue or aspirate – the proportion of aneuploid tumors was 18/25 in tissue samples and 9/13 in aspirates in the high-grade malignant sarcomas (Table 1).

Combining the data from the analysis of the preoperative needle aspirates, 13 tumors diagnosed as sarcomas and one as a malignant unspecified tumor were aneuploid, and all 14 were histopathologically high-grade malignant in the aspirates. However, the number of low-grade sarcomas was too small for evaluation of the relationship between ploidy and malignancy grade.

## Discussion

In 26 out of our 29 tumors, the aspirates permitted flow cytometric DNA analysis. The three failures

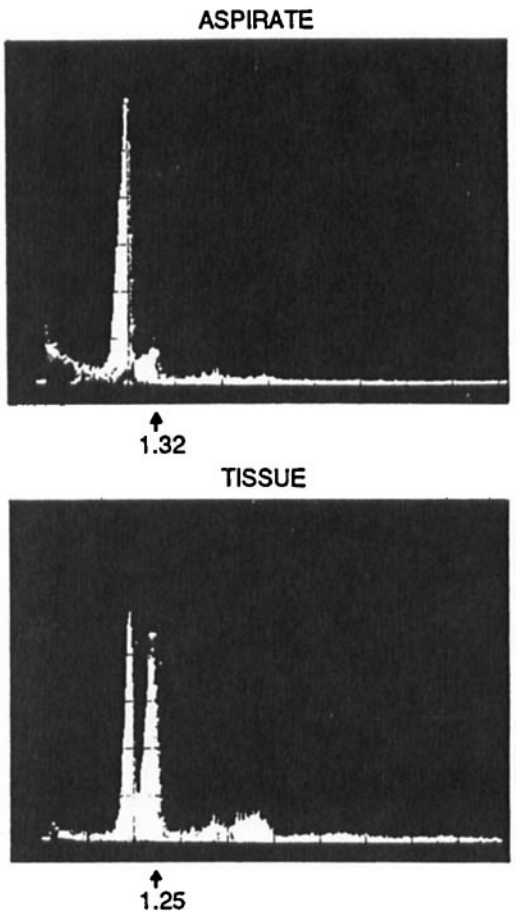


Figure 2. DNA distribution of the single cell analysis of aspirate and tissue sample from a liposarcoma Grade IV (Case 21). DI values of aneuploid cell populations are indicated.

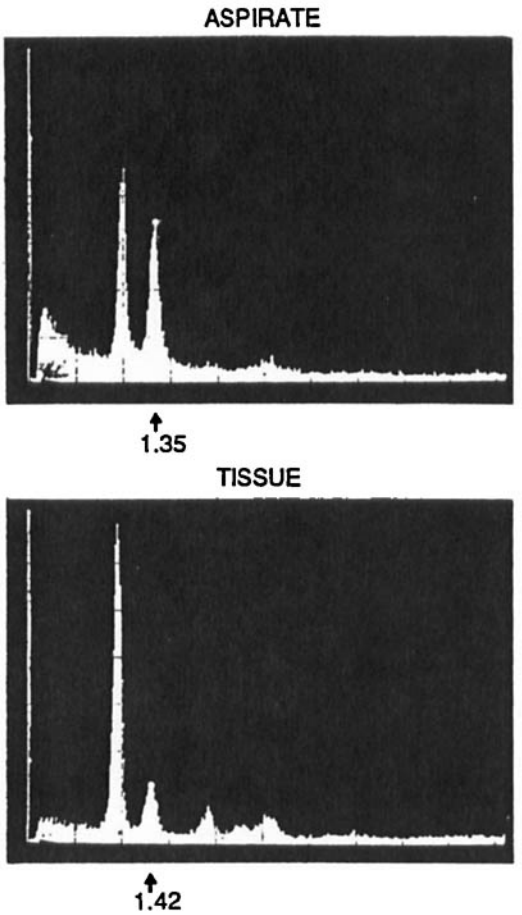


Figure 3. DNA distribution of the single cell analysis of aspirate and tissue sample from a leiomyosarcoma Grade III (Case 10). DI values of aneuploid cell populations are indicated.

– too few cells for analysis – were probably in part due to the sampling technique, because the cells for the flow analysis consisted of those remaining in the needle and syringe after ordinary smearing for cytodiagnosis had been performed. Two of them were benign soft tissue tumors, one a lipoma, and one a proliferative fasciitis. Aspirates from lipomas usually consist of small tissue fragments or clusters of large fat cells (Walaas & Kindblom 1985). These small fragments are in most cases enough for a cytodiagnosis, but the number of cells is small. Earlier experience has also shown that it might be difficult to obtain material for cytodiagnosis from fibromatous tumors rich in intercellular substance (Åkerman et al. 1985). To enhance the cellular yield for flow analysis, one or two aspirates from which all the material is flushed out for DNA analysis should be performed.

There was a good correspondence between the cytologic and histologic diagnoses, particularly with respect to sarcoma. There was also a good correlation between the DNA values obtained from analysis of aspirates and tissue samples, respectively. Thus, the same ploidy classes were found in 20/26 cases. Among the 6 remaining cases, sampling error at aspiration may explain the difference in 2 cases, but we have no explanation for the others. One might speculate on the presence of subpopulations of tumor cells with different DNA values in different parts of the tumor as described in osteosarcoma (Büchner et al. 1985). On the other hand, we found very similar DNA values in all 11 soft tissue sarcomas

where tissue samples were collected from different parts of the tumor. Another explanation for DNA discrepancies might be selection or destruction of certain tumor cells at aspiration and flushing.

Correlations have been found between ploidy and clinicopathologic malignancy grading in a variety of musculoskeletal tumors (Kreicbergs et al. 1980, 1982, Keen et al. 1985, Mankin et al. 1985), but a prognostic value of DNA analyses for survival has so far only been reported in chondrosarcomas (Kreicbergs et al. 1980). However, if DNA analyses turn out to add prognostic information to the variables now used, then, knowledge about the DNA pattern should be of importance for planning of the treatment of other musculoskeletal tumors than chondrosarcoma.

We found a good correspondence in three fourths of the cases between DNA ploidy in preoperative aspirates and in tissue specimens, and the combination of sarcoma diagnosis, and aneuploidy in aspirates corresponded to aneuploid high-grade malignant sarcomas in tissue samples.

## References


Büchner T, Hiddemann W, Wörmann B, Kleinemeier B, Schumann J, Göhde W, Ritter J, Müller K M, von Bassewitz D B, Roessner A, et al. Differential pattern of DNA aneuploidy in human malignancies. Pathol Res Pract 1985;179(3):310–7.

Ewers S B, Långström E, Baldetorp B, Killander D. Flow cytometric DNA analysis in primary breast carcinomas and clinicopathological correlations. Cytometry 1984;5(4):408–19.

Hiddemann W, Schumann J, Andreef M, Barlogie B, Herman C J, Leif C, Mayall B H, Murphy R F, Sandberg A A. Convention on nomenclature for DNA cytometry. Committee on Nomenclature, Society for Analytical Cytology. Cytometry 1984;5: 445–6.

Keen M E, Clevenger C V, Williams T J, Murad T M, Epstein A L, Bauer K D. Correlation of two color flow cytometric evaluation of smooth muscle tumors with histopathologic and clinical parameters. Am J Clin Pathol 1985;83:401.

Kreicbergs A, Broström L A, Cewrien G, Einhorn S. Cellular DNA content in human osteosarcoma: aspects on diagnosis and prognosis. Cancer 1982;50(11):2476–81.

Kreicbergs A, Zetterberg A, Söderberg G. The prognostic significance of nuclear DNA content in chondrosarcoma. Anal Quant Cytol 1980;2(4):272–9.

Mankin H J, Connor J F, Schiller A L, Perlmutter N, Alho A, McGuire M. Grading of bone tumors by analysis of nuclear DNA content using flow cytometry. J Bone Joint Surg (Am) 1985;67(3):404–13.

Markhede G, Angervall L, Stener B. A multivariate analysis of the prognosis after surgical treatment of malignant soft tissue tumors. Cancer 1982;49(8): 1721–33.

Rydholm A, Berg N O, Gullberg B, Thorngren K G, Persson B M. Epidemiology of soft tissue sarcoma in the locomotor system. A retrospective population based study of the interrelationships between clinical and morphologic variables. Acta Pathol Microbiol Immunol Scand (A) 1984;92(5):363–74.

Rydholm A, Rööser B, Persson B M. Primary myectomy for sarcoma. J Bone Joint Surg (Am) 1986;68(4):586–9.

Stener B. Surgical treatment of soft tissue tumors. In: International Cancer Congress 12, Buenos Aires 1978, Advances in medical oncology, research and education, Vol. 10, Clinical cancer principal sites 1. (Ed. Kumar S) Pergamon Press, Oxford, New York 1979:147–56.

Tribukait B, Gustafson H, Esposti P. Ploidy and proliferation in human bladder tumors as measured by flow cytofluorometric DNA analysis and its relations to histopathology and cytology. Cancer 1979;43(5): 1742–51.

Vindelov L L. Flow microfluorometric analysis of nuclear DNA in cells from solid tumors and cell suspensions. A new method for rapid isolation and straining of nuclei. Virchows Arch (Cell Pathol) 1977;24(3):227–42.

Walaas L, Kindblom L G. Lipomatous tumors: a correlative cytologic and histologic study of 27 tumors examined by fine needle aspiration cytology. Hum Pathol 1985;16(1):6–18.

Zajicek J. Aspiration biopsy cytology. P.1 Cytology of supradiaphragmatic organs. Monographs in clinical cytology. Karger, Basel 1974;4.

Åkerman M, Rydholm A, Persson B M. Aspiration cytology of soft tissue tumors. The 10 year experience at an orthopedic oncology center. Acta Orthop Scand 1985;56(5):407–12.


## Acknowledgements

This work was supported by grants from the Swedish Medical Research Council, John and Augusta Persson Foundation for Scientific Medical Research, and Stiftelsen för bistånd åt vanföra i Skåne.