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Assessment of Proliferative Activity in Soft Tissue Sarcomas Showing PCNA and Ki-67 Reactivity Immunohistochemically

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Abstract: The aim of this study was to determine PCNA (Proliferating Cell Nuclear Antigen) and Ki-67 expression of various soft tissue sarcomas (STS) immunohisto-chemically and to correlate with the histologic grade of these tumors. Retrospectively 185 cases of STS were re-examined. Of the 185 cases, 35 primary STS cases which were localized in the extremities and trunk wall were included in this study. Anti-PCNA and Anti-Ki-67 monoclonal antibodies were applied to the paraffin sections of tumor tissues immunohistochemically. A semiquantitative score was employed to assess the percentage of cells that were positively stained. The comparison of the positively stained tumor cell proportion and the histologic grade of the tumor was determined using PCNA (≤ 25%, >25%) and Ki-67 (≤ 10%, > 10%) indices. A positive correlation was found between increasing PCNA and Ki-67 expression and histologic grade and metastasis.

Our results indicate that PCNA and Ki-67 can be used as a prognostic factor in STS.

Key Words: Soft tissue sarcomas, PCNA, Ki-67, immunohistochemistry.

Introduction

STSs exhibit a wide spectrum of biological behavior. This variety complicates the determination of prognoses and the treatment in tumors which are similar morphologically. In recent years, many multivariate analyses introducing clinical prognostic features have shown that the histological grade was the most important and reliable prognostic factor for STS (1,2).

The grade of malignancy is determined by a combined assessment of several histological features: mitotic activity (frequency and abnormality of mitotic figures), necrosis, cellularity, cellular pleomorphism or anaplasia (3). However, various technical artefacts restrict the determination of these criteria crucially and sensitively. Immunohistochemical methods of assessing cell proliferation are now becoming popular. In recent years, many types of monoclonal antibodies which are useful for the demonstration of cell cycle related antigens immunohistochemically have been defined (4,5).

In the literature some studies have shown a correlation between an abundant expression of PCNA and Ki-67 and unfavorable prognosis in various (cerebral tumors, breast cancer, malignant lymphoma, STS) malignancies (5-7). Experience of PCNA and Ki-67 expression in STS is, however, relatively limited.

The present study examines the relationship between PCNA, Ki-67 expression and histologic grade of various types of STS.

Materials and Methods

Patients: There were 185 patients who were treated for primary STS at Çukurova University Faculty of Medicine 1988-1995. All of these cases of STS were re-examined retrospectively from the files of the Pathology department. Of these patients 110 (59.5%) were men, 75 (40.5%) were women with a median age of 40.2 years (range 3/12- 85) (The age of 12 patients was not known).

For this study we selected 35 cases of primary STS. For the selection of cases we took the following into consideration: patients with a STS of the extremity and trunk wall, patients with clinical follow-up, tumors which are different histological types, the quality of tumor tissue for immunohistochemistry. In the study group,

there were 20 (57.2%) men and 15 (42.8%) women with a median age of 44.7 years (range 7- 73) (The age of one patient was not known). The mean tumor size was 14.6 (range 5-30) cm (information on the tumor size was not available in seven cases). Twenty-three (65.7%) tumors were in the lower extremity, four (11.4%) were in the upper extremity and eight (22.8%) were located on the trunk wall. Among the patients who had follow-up, six developed metastases (to lungs in five cases, to humerus in one case) and three developed recurrence. In three cases no metastases or recurrence were established during the follow-up of 4 years. The median follow-up time for survivors was 2.4 years (range 1-8).

Histotype and malignancy grade: Histologic grading and classification were made based on the criteria of Enzinger and Weiss (3). For identification of mitoses, the number of mitoses per ten high-power fields (HPF) (x400) were counted separately by two pathologists. Mitotic counts were done in the most cellular and mitotic areas. Pyknotic nuclei and suspicious cells were excluded. Cellularity was estimated and categorized as high, intermediate and low. In the term of necrosis, all of the hematoxylin and eosin stained slides with tumor for each case were examined. The cases were classified into two groups by each pathologist: less than 50% tumor necrosis for all the examined tumor surface and tumor necrosis on more than half of the examined tumor surface.

Immunohistochemistry: Immunostaining was performed by the avidin-biotin peroxidase technique (ImmuStain Strept A-B Universal Kit, DPC/USA).

Negative and positive control slides were used for each study group.

Evaluation of proliferative activity: A semiquantitative score was employed to assess the percentage of cells that were positively stained with PCNA and Ki-67 (8,9). The entire specimen area of each slide examined separately by two pathologists (ÖA, CE) on two separate occasions at two week intervals randomly without knowledge of the code. Briefly, the most cellular and markedly stained parts of the tumors for each slide were determined on low-power field. To determine the percentage of positive cells for PCNA and Ki-67, at least 200 cells were counted in two separate HPFs (x400) per slide. A mean of the two percentages was calculated for each case and was considered the staining index. In cases where significant field-to-field variation in the percentage of positive cells existed, another HPF was examined and a mean of the three percentages was calculated. The percentage of PCNA and Ki-67 positive cells was classified as ≤10%, 11-25%, 26-50%, >50%. All immunostained nuclei, independent of intensity, were scored as positive. The germinal center cells of human tonsil which stained positively for PCNA and Ki-67 were employed as positive controls. All of the immunostained slides were evaluated under a light microscope (Nikon, Optiphot-2).

Statistical analysis: Differences between categorical variables were determined using the chi-square test and the SPSSPC program was used for statistical analysis.

Results

The distribution of histologic types and subtypes of 35 cases of STS are shown in Table 1. The number of mitoses per 10 HPF ranged from two to 40 (median 9.3) in the tumors of the study group. Tumor necrosis was absent in 12 (34.3%) tumors, was less than 50.0% for all the examined tumor surface in 14 (40.0%) tumors and on more than half of the examined tumor surface in nine (25.7%) tumors. Cellularity was high in 23 (65.8%), intermediate in seven (20.0%), and low in five (14.2%). Based on these criteria 16 (45.7%) tumors were considered overall to be histologic Grade III, 12 (34.3%) were Grade II, 7 (20.0%) were Grade I. In cases with metastasis, one tumor was Grade II and five were Grade III. Three cases with recurrences were Grade I, II and III. The pattern of nuclear staining was usually granular with PCNA and predominantly diffuse-homogeneous with Ki-67. Some mitotic cells fail to show any staining especially with PCNA. Rarely cytoplasmic staining with Ki-67 is observed. The positivity for PCNA three (10.0%) cases were associated with ≤10%, six (20.0%) were 11-25%, 15 (50.0%) were 26-50% and six (20.0%) were >50%. Tumor cells failed to show any PCNA staining in five cases. Four cases were Grade I, and one was Grade II in the PCNA negative group. The positivity for PCNA was 11.9% in Grade I tumors, 32.0% and 39.6% in Grade II (Fig 1,2) and Grade III tumors (Table 2).

In one case with recurrence the PCNA index was 50.5%. A cut-off point score of 25% was used for the PCNA index. The differences between tumor stages and PCNA groups was found to be statistically significant (p=0.008). This significance was related to Grade III tumors (Table 3).

The positivity groups of Ki-67 were four (16.6%) cases for ≤10%, five (20.8%) for 11-25%, 12 (50.0%) for 26-50% (Figs 3,4), three (12.6%) for >50%. There was no Ki-67 staining in 11 tumors. Five cases were Grade I, one was Grade II and five were Grade III in the

Histologic Types and Subtypes	Number	%
Malignant Fibrous Histiocytoma	8	22.8
-Storiform-pleomorphic	6	
-Inflammatory	1	
-Giant cell	1	
Liposarcoma	8	22.8
-Myxoid	3	
-Pleomorphic	1	
-Round cell	1	
-Mixed	3	
Rhabdomyosarcoma	7	20.0
-Alveolar	3	
-Embryonal	1	
-Pleomorphic	3	
Malignant Peripheral Nerve Sheath Tumor	4	11.5
Leiomyosarcoma	3	8.5
Synovial sarcoma	2	5.8
Fibrosarcoma	2	5.8
Malignant Mesenchymoma	2	1.0
Malignant Hemangiopericytoma	3	1.7
Angiosarcoma	1	2.8
TOTAL	35	100.0

Table 1. Distribution of histologic types and subtypes of 35 cases of STS.
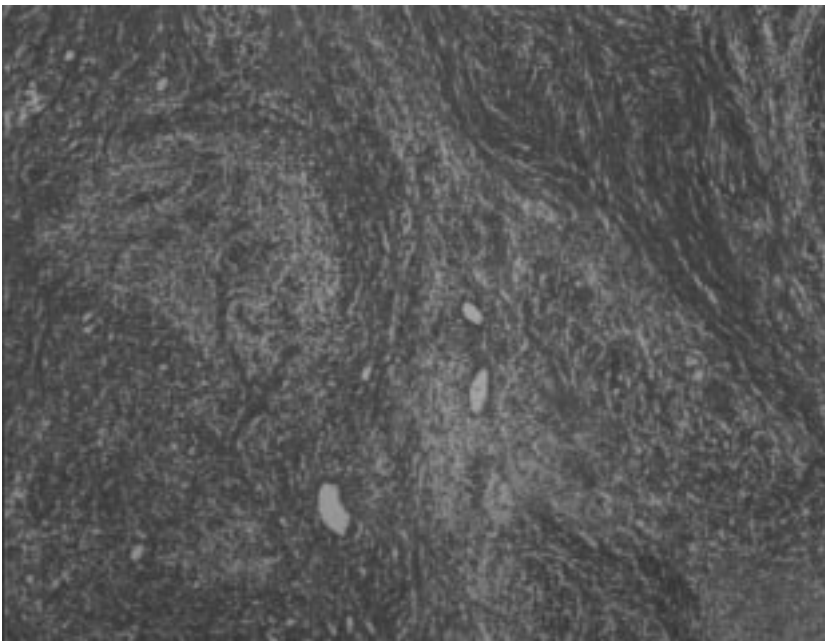

Figure 1. Malignant peripheral nerve sheath tumor, Grade II (HEx40).

Ki-67 negative group. The Ki-67 positivity was 7.2% in Grade II and 39.2% in Grade III tumors (Figs 5,6) (Table 4). For Ki-67 staining a significant difference was observed between Grade I and Grade II-III tumors when there was no significant difference between Grade II and III tumors. There was 23.7% positivity for Ki-67 in one

Tumor Grade	PCNA-Positive Cells (%)				TOTAL (%)
	≤ 10	11-25	26-50	>50	
I	1	2	-	-	3 (10.0)
II	1	3	5	2	11 (36.6)
III	1	1	10	4	16 (53.3)
TOTAL (%)	3 (10.0)	6 (20.0)	15 (50.0)	6 (20.0)	30 (100.0)

Table 2. Distribution of grade and positivity of 30 cases of STS stained with PCNA.

Tumor Grade	PCNA index (%)		TOTAL (%)	P
	≤ 25	> 25		
I	3	0	3 (10.0)	
II	4	7	11 (36.6)	0.008
III	2	14	16 (53.3)	
TOTAL (%)	9 (30.0)	21 (70.0)	30 (100.0)	

Table 3. Relationship between PCNA index and tumor grade.

Tumor Grade	Ki-67 Positive Cells (%)				TOTAL (%)
	≤ 10	11-25	26-50	> 50	
I	2	-	-	-	2 (8.3)
II	2	4	5	-	11 (45.8)
III	-	1	7	3	11 (45.8)
TOTAL (%)	4 (16.6)	5 (20.8)	12 (50.0)	3 (12.6)	24 (100.0)

Table 4. Distribution of grade and positivity of 24 cases of STS stained with Ki-67.
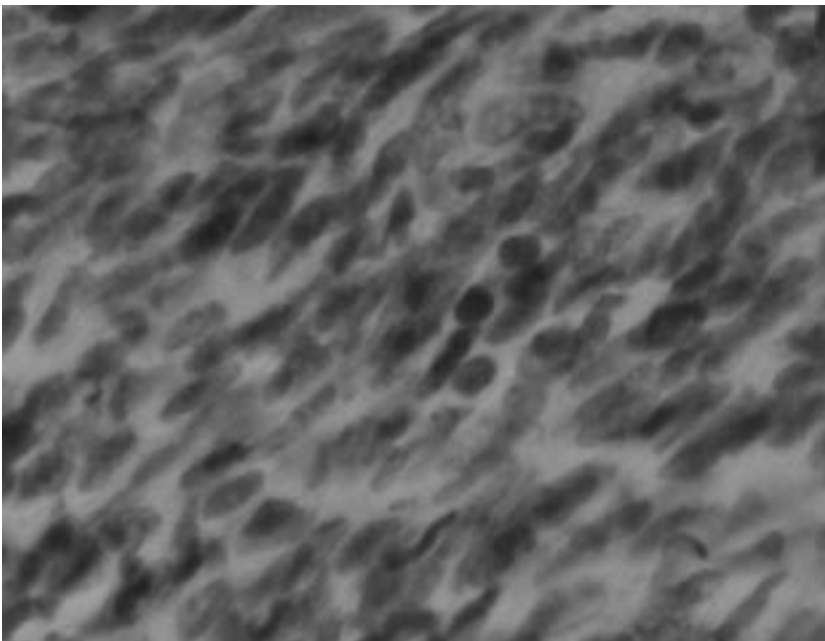

Figure 2. Grade II Malignant peripheral nerve sheath tumor with low numbers of PCNA staining cells (x200).

of the cases with recurrences. A cut-off point score of 10% was used for the Ki-67 index as used in some former studies (8,9). We found the difference between the Ki-67 index and tumor stages group to be statistically significant (p=0.002) and this significance was related to Grade III tumors (Table 5). The Ki-67 index was >10% in six (100%) cases with metastasis and in 14 (77.7%) cases without metastasis. For PCNA the index was >25% in five (83.3%) cases with metastasis and in 16 (66.6%) cases without metastasis. The difference was not statistically significant between the groups.

Discussion

Proliferation and cellular differentiation are inversely related processes (8). There is a strong correlation between the proliferation rate of tumors and clinical outcome. The prognostic importance of the frequency of mitosis as shown in several studies indicates that further investigation of the cell proliferation in patients with STS is required (5,10,11). Furthermore, some authors (12) reported that mitotic counts are not completely reliable or reproducible, they only reflect one part of the cell cycle (the mitotic or M-phase). PCNA and Ki-67 are two cell cycle antigens which are expressed in proliferative states and they can be demonstrated in tissues using monoclonal antibodies. In the literature a good correlation has been shown between PCNA and Ki-67 reactivity and prognoses in different tumors (carcinomas of the breast, lung, cervix, colon, as well as malignant lymphomas, brain tumors and sarcomas) (6,7,13). A significant correlation has been reported between Ki-67 expression and mitotic rate in STS (14).

A correlation between PCNA and Ki-67 reactivity and tumor grade in STS has been detected in different retrospective studies (8,15,16). Sahin and co-workers

Tumor Grade	Ki-67 index (%)		TOTAL (%)	P
	≤ 10	> 10		
I	2	0	2 (8.3)	
II	2	9	11 (45.8)	0.002
III	0	11	11 (45.8)	
TOTAL (%)	4 (16.6)	20 (83.3)	24 (100.0)	

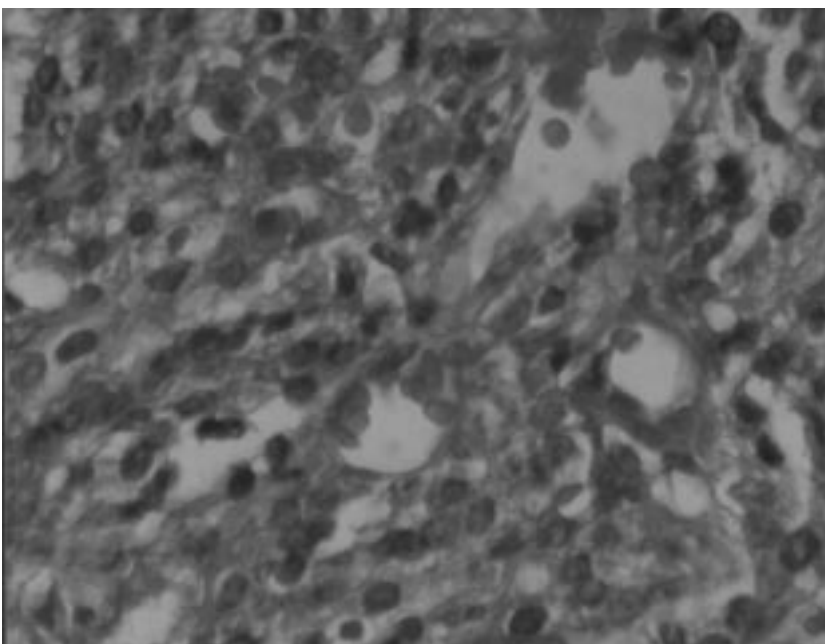Table 5. Relationship between Ki-67 index and tumor grade.
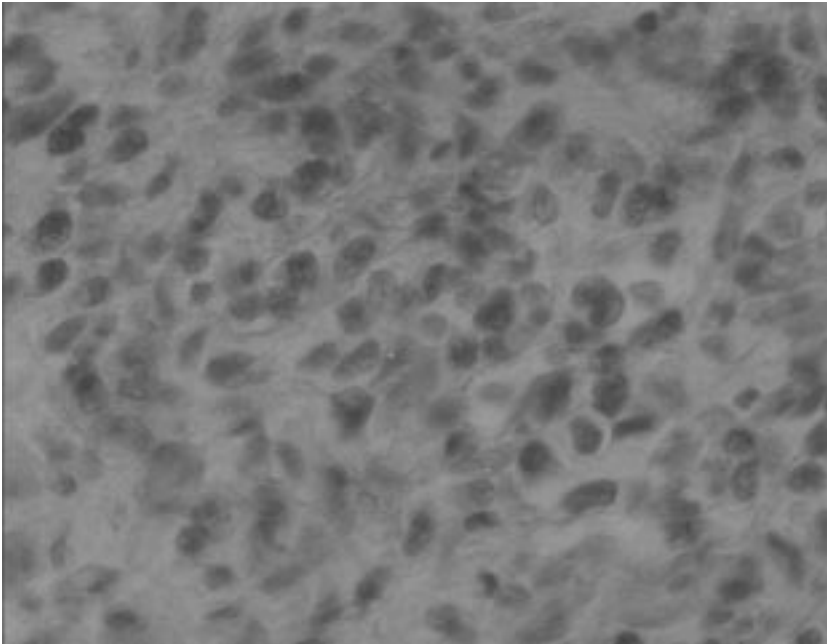

Figure 3. Angiosarcoma, Grade II (HEx 400).

Figure 4. Grade II Angiosarcoma with numerous Ki-67 staining cells indicating high proliferation (x400).
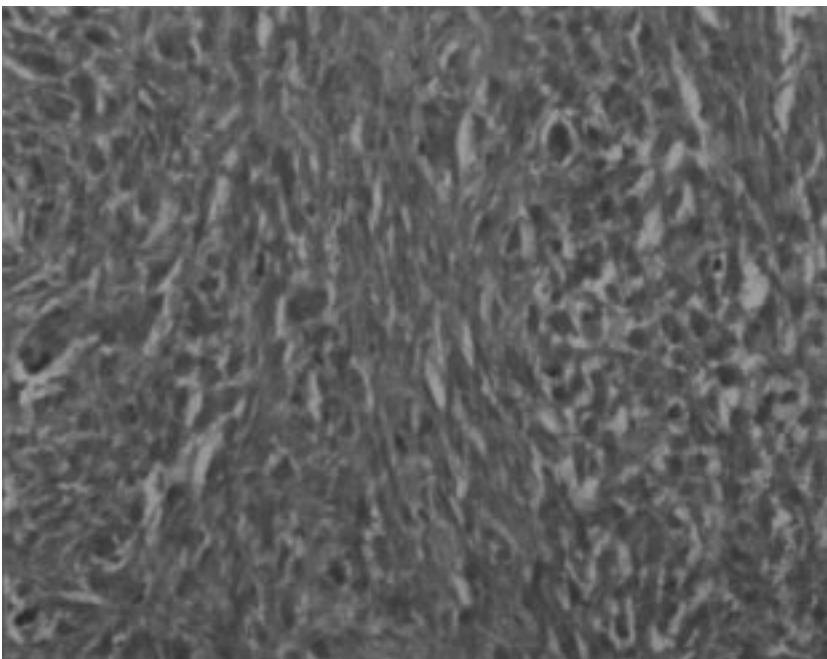

Figure 5. Grade III Malignant fibrous histiocytoma (HEx100).

(17) have reported that the Ki-67 index is low in benign and Grade I tumors, and high in Grade II,III tumors. Heslin et al. suggested that Ki-67 score is an independent prognostic molecular marker that predicts distant metastasis and tumor mortality (18).

In the present study we also demonstrated a positive correlation between Ki-67 and PCNA indices and histologic grade. PCNA index was ≤25% and Ki-67 was ≤10% in all of the Grade I tumors (100%). In Grade III tumors, the PCNA index was >25% in 87.5% and the Ki-
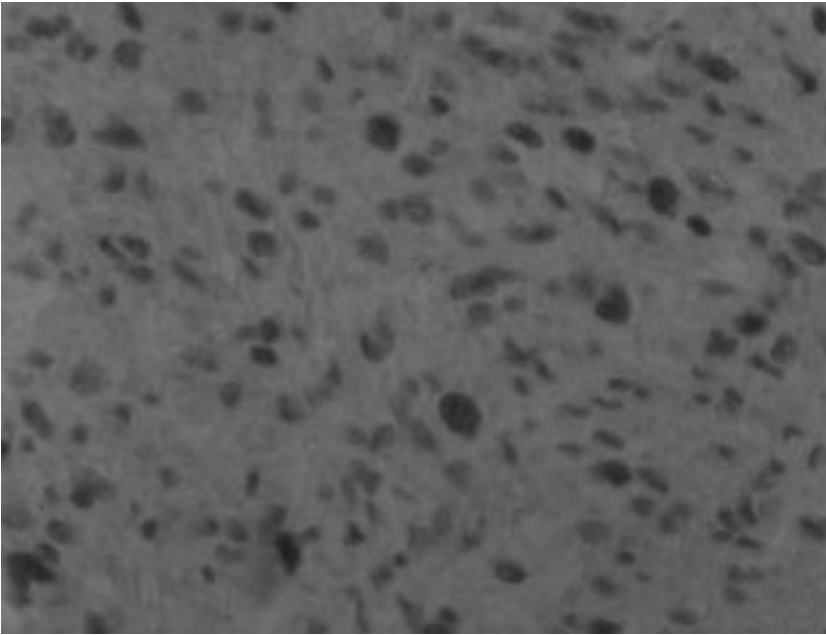
Figure 6. Grade III Malignant fibrous histiocytoma with Ki-67 index >10 % (x200).

67 index >10% in 100% of tumors. We observed disparity between Ki-67 and PCNA indices in five tumors. High PCNA- low Ki-67 indices were demonstrated in four of these tumors. In previous studies (9) a lack of correlation between PCNA and Ki-67 expression has been shown which they attributed to the relatively long half-life of PCNA, methodological problems, histotype specificity, and inter- and intra-tumoral heterogenity. We observed low PCNA, high Ki-67 index in one of the Grade III malignant fibrous histiocytoma (MFH). Choong and Åkerman (9) have also surprisingly demonstrated a lack of correlation between PCNA expression and tumor grade in their series (n = 185). However, in the study of Dreinhöfer et al. (19) low PCNA indices have been found in some of the Grade IV -MFH cases with metastases. Hall et al. noted that the expression of PCNA may be deregulated in some tumors. Furthermore, Choong and Åkerman suggested that combining both PCNA and Ki-67 indices as a marker of proliferation is better than with either index alone (9). Tumor cells fail to show any PCNA or Ki-67 staining in some cases. In our study, 80.0% of PCNA negative tumors were Grade I. There was no staining with Ki-67 in 45.4% of the Grade III tumors. Two of these cases were incisional biopsy and the tissue samples were small. Brown (12) noted that most tumors consist of a heterogeneous cell population within which there are different proliferations and thus, Ki-67 staining on a small biopsy may not reflect the predominant proliferation rate of a tumor.

Swanson et al. (20) have also observed a disparity between Ki-67 score and histologic grade in some tumors (synovial sarcoma, leiomyosarcoma, MFH) in their series. In the same study they noted that there was a similar disparity in the literature. In our series when the Ki-67 index was 8.0% in one of the Grade II synovia lsarcomas, there was no staining in the other. The immunostaining has not been observed in some of the mitotic cells, especially with PCNA. Similarly, in a previous study an unexplained negativity was observed in the mitotic cells and the possibility was considered that this was related to the artefacts of fixation or processing (4).

In many different tissues, including cervical carcinoma, lung carcinoma, breast carcinoma the cytoplasmic staining with Ki-67 has been reported. But the reason for this is not clear. It may represent a cross-reaction with an unrelated cytoplasmic epitope or a genuine detection of the Ki-67 antigen (12).

The histologic type and subtype may be used as a short-cut to establish the tumor grade. It is accepted that some of the histologic types and subtypes are high-grade, and some are low-grade directly (3). In the literature there are also several studies which evaluated the proliferative activity of histologic subtypes (19). But because of the lack of the number of cases we did not compare histologic subtypes with PCNA and Ki-67 reactivity. In the present study, high PCNA and Ki-67 indices were observed in the cases with metastases and a correlation was detected between the proliferation indices

and the presence of metastases. The PCNA index was >25% in the 83.3 % and the Ki-67 index was >10% in 100% of cases with metastases. Choong et al. have also observed higher PCNA and Ki-67 indices in 48 MFH cases with metastases (8,9). There was a reliable clinical follow-up in only 12 cases of the study group.

Finally, we suggest that immunohistochemical assessment of cell proliferation is more practical and easy than the other techniques and may yield better information in the current prognostic models for STS.

And the data will be more concrete when PCNA and Ki-67 values are evaluated by combining the biological behaviour of tumors in large series with clinical follow-up.

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