

Prognostic Significance of Proliferating Cell Nuclear Antigen Expression in Soft Tissue Sarcomas of the Extremities

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= Abstract = We analysed 40 patients with soft tissue sarcomas (STS) of the extremities to investigate the prognostic significance of the growth fraction of the tumor. The patients consisted of 27 males and 13 females, and the ages ranged from 18 to 70 years (median 46.9 years). Tumors were located in the thigh (47.5%), forearm (15%), buttock (10%), shoulder (10%), axilla (5%), arm (5%), lower leg (5%), and foot (2.5%) in a descending order of frequency. Histologically, malignant fibrous histiocytoma (25%) and liposarcoma (25%) were the most common types, followed by synovial sarcoma (17.5%), malignant schwannoma (10%), and rhabdomyosarcoma (5%). Local recurrence ranging from 1 to 4 times (average 1.7 times) was noted in 18 cases (45%). Distant metastasis was found in 5 out of 30 patients (16.7%), and the lung, liver, and abdominal wall were the predilection sites. Cumulative survival rates of all STS studied were 76% and 58% in 2 years and 5 years, respectively. Univariate analysis revealed that patient's age, surgical stage, histologic grade, differentiation of tumor cells, mitotic count, and proliferative index were related to survival. Among known parameters of histologic grading, only mitotic count was correlated with proliferative index. Multivariate analysis showed that patient's age, surgical stage and proliferative index were significant prognostic factors. This study indicates that the proliferative index using the antibody against proliferating cell nuclear antigen is useful to assess the prognosis of STS.

Key Words: Sarcoma, Grade, Proliferating cell nuclear antigen

INTRODUCTION

Soft tissue sarcomas (STS) comprise a heterogeneous group of tumors and histologic

type alone cannot provide a definite conclusion to estimate the prognosis of the patients, although it could be one of the important prognostic factors. Major reasons for this might be the relative rarity of STS, discrepancies in diagnosis and classification even among experienced pathologists because of the complex histology of STS, and the lack of a

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united view on the terminology (Tsujiimoto *et al.* 1988). These recognitions have led a number of clinical investigators to propose the grade of malignancy as an important prognostic factor (Suit *et al.* 1973; Russell *et al.* 1977; Markhede *et al.* 1982; Myhre-Jensen *et al.* 1983 & 1991; Costa *et al.* 1984; Trojani *et al.* 1984). However, it is difficult to define an universally accepted histologic grading system. Broders *et al.* (1939) outlined the grade of STS by several histologic features including degree of cellularity, cellular pleomorphism or anaplasia, mitotic activity (frequency and abnormality of mitotic figures), degree of necrosis, and growth pattern (Enzinger and Weiss 1988). Although absolute criteria to determine an accurate prognosis of the patients with STS have not been established, the following factors have been suggested; cellularity, differentiation, pleomorphism, mitosis, and necrosis. Among them, mitosis and necrosis have been regarded as the most important parameters (Enzinger and Weiss 1988), and the reproducibility of grading system showed relatively high agreement (Coindre *et al.* 1986).

There has been a suggestion that antibodies against proliferating cells such as Ki-67 might be used for the purpose of the investigation of the cell proliferation, which might provide a more accurate estimate of the prognosis (Tsujiimoto *et al.* 1988). Recently, a new antibody against proliferating cell nuclear antigen (PCNA) has been developed (Celis and Celis 1985; Robbins *et al.* 1987; Garcia *et al.* 1989) and has proved to be useful in assessing the growth fraction of the tumor. We studied 40 cases with STS of the extremities to investigate prognostic factors related to patient's survival and tested the usefulness of proliferative index using the antibody against PCNA.

MATERIALS AND METHODS

STS of the extremities were obtained from 40 patients by surgical resection in the Department of Surgery, Seoul National University Hospital during the period from January 1980 to

June 1991. We excluded STS arising from bone, internal organs, retroperitoneum, head and neck as well as the cases with inadequate clinical information, poor tissue preparation, and insufficient tissue sectioning. Clinical data including age, sex, location, size, stage, local recurrence, distant metastasis, and therapeutic modalities were obtained from the medical records and the cases have been followed up for more than 16 months.

For the light microscopic examination, tissue samples were fixed in 10% formalin for 24 hours, processed routinely, and embedded in paraffin. Paraffin-embedded tissues were cut into 4 μ m-thick sections and were stained with hematoxylin-eosin. We used the grading system proposed by Trojani *et al.* in 1984, and summarize in Table 1.

For the immunohistochemical study against PCNA, we used the monoclonal antibody, PCNA (DAKO) followed by avidin-biotin peroxidase complex method using Vectastain (Vector). Proliferative index (PI) was measured by the formula described below. The cells were counted in the best stained area at the high power field ($\times 400$).

Proliferative index (PI)

$$= \frac{\text{PCNA (+) tumor cell nuclei}}{100 \text{ tumor cell nuclei}} \times 100(\%)$$

Survival curves were determined from the time of the adequate surgery and were plotted using the Kaplan-Meier method. Then the log-

Table 1. Grading system for the soft tissue sarcoma (Trojani *et al.* 1984)

Score	0	1	2	3
Differentiation		Like normal	Certain	Undifferentiated
Mitosis (/10HPF)		0-9	10-19	20-
Necrosis	No	<50%	50%	

Grade I: 2-3

Grade II: 4-5

Grade III: 6-8

rank test was used to evaluate the significance of individual factors using the PC-SAS software, 6.04 version. The relation between the proliferative index and parameters of histologic grading was estimated by Kendall's tau B correlation coefficients. We used this method for the purpose of the analysis of collinearity before the multivariate analysis. Multivariate analysis was taken by Weibull model.

RESULTS

The patients consisted of 27 males and 13 females, and ages ranged from 18 to 70 years (median 46.9 year). But the peak of age distribution was located in the fifth decade. Anatomical locations of STS were 77.5% in proximal and 22.5% in distal. Details were as follows; thigh (47.5%), forearm (15%), buttock (10%), shoulder (10%), axilla (5%), arm (5%), lower leg (5%), and foot (2.5%) in a descending order of frequency. Maximal diameters of STS were mostly less than 10 cm (72.5%). Proportions of patients' stages were 38.9% in stage 1, 22.2% in stage 2, 30.6% in stage 3 and 8.3% in stage 4. Local recurrence ranging from 1 to 4 times (average 1.7 times) was noted in 18 cases (45%). Distant metastasis was found in 5 out of 30 cases (16.7%), and the lung, liver and abdominal wall were the predilection sites. Detailed clinical information is described in a previous report (Chung *et al.* submitted).

Histologically, malignant fibrous histiocytoma (25%) and liposarcoma (25%) were the most common types, followed by synovial sarcoma (17.5%), malignant schwannoma (10%), rhabdomyosarcoma (5%) and others (17.5%). We divided STS into grade I (44.8%), grade II (27.6%) and grade III (27.6%) by Trojani's grading system. But the parameters were reassessed for the statistical examination. Differentiation of tumor cells was divided into well differentiated group (62.0%) consisting of Trojani's score 1 and 2, and poorly differentiated group consisting of Trojani's score 3 (38.0%). Mitotic count of the tumor was divided into low

mitotic count (less than 10/HPF) (62.0%) consisting of Trojani's score 1, and high mitotic count (equal to or more than 10/HPF) (38.0%) consisting of Trojani's score 2 and 3. Extent of necrosis divided into absence of necrosis (72.4%), present but less than 50% necrosis (24.1%), and present but equal to or more than 50% necrosis (3.5%).

Immunohistochemical stain against PCNA showed coarse granular reactivity in the nuclei (Fig. 1). Average proliferative index was 47% and we divided STS into high PI (equal to or more than 55%) (77.3%) and low PI (less than 55%) (22.7%).

Of the forty patients, twenty two patients have been alive, fifteen patients were dead, and three patients were lost during the clinical follow-up. Cumulative survival rates of all STS studied were 76% and 58% in 2 years and 5 years, respectively. Univariate analysis of the survival curve revealed that the patient's age, surgical stage, histologic grade including differentiation of tumor cell and mitotic count, and proliferative index were of prognostic importance (Fig. 2). Other parameters such as local recurrence, sex, size, histologic diagnosis, and location did not show any prognostic significance. Among known parameters of histologic grading, only mitotic count was correlated with proliferative index by Kendall's tau B coefficients ($p < 0.05$). Multivariate regression analysis using the Weibull model showed that patient's age, surgical stage and proliferative index could be independent prognostic factors (Table 2).

DISCUSSION

The role of the pathologist in the management of STS is in making a correct diagnosis and expressing the characteristics of the tumor in such a way that the management team has a clear understanding of its biological potential (Worth 1988). To evaluate the behavior of a STS, one needs to know the cell type, grade, mitotic count, size, depth and location.

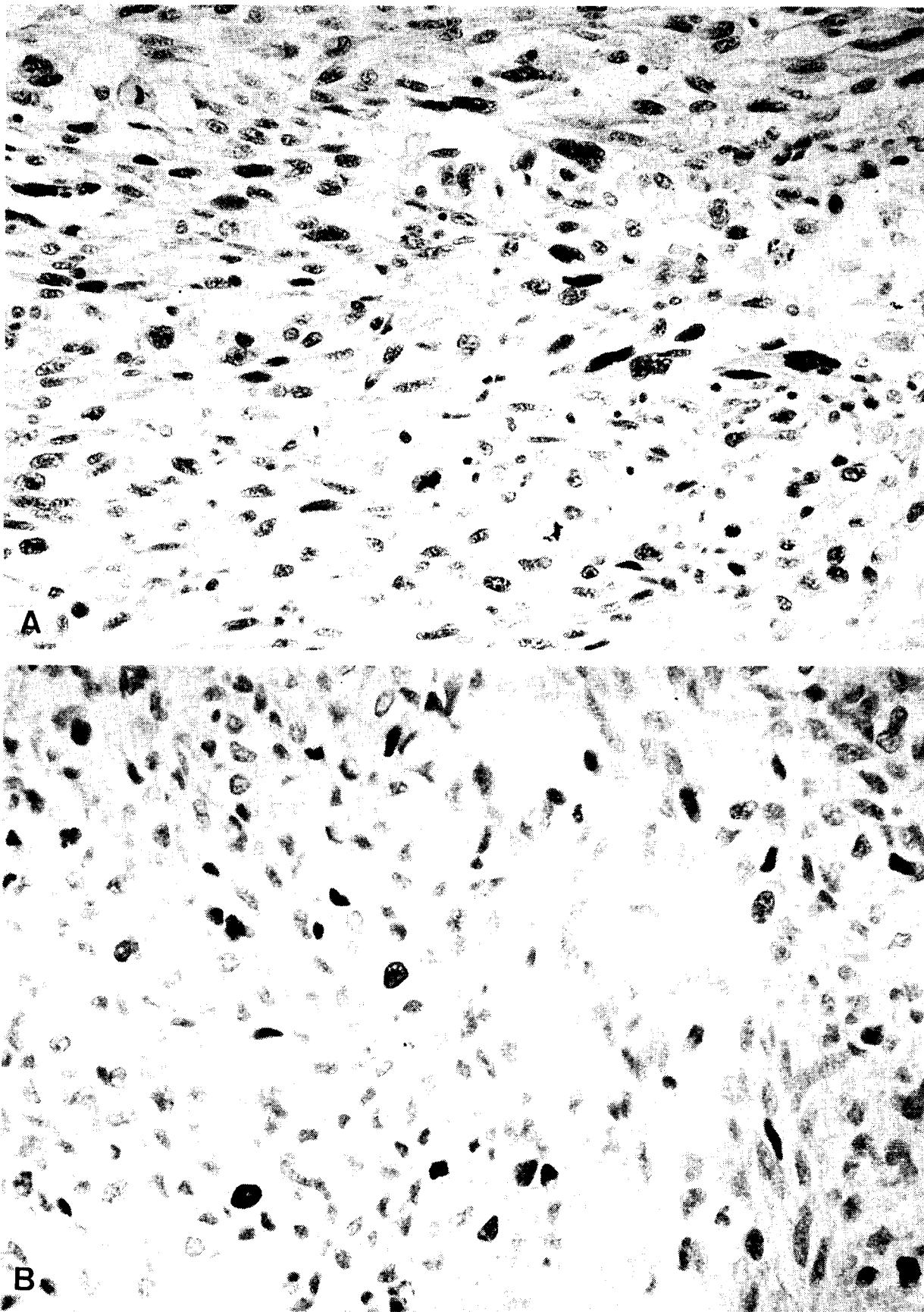


Fig. 1. Immunohistochemical stain against PCNA shows coarse granular reactivity in the tumor cell nuclei with (A) high proliferative index (PI) and (B) low PI.

Generally, mitotic count has been used for the evaluation of the patient's prognosis with or without other factors such as differentiation,

cellularity and necrosis (Markhede *et al.* 1982; Myhre-Jensen *et al.* 1983 & 1991; Costa *et al.* 1984; Trojani *et al.* 1984). However variable

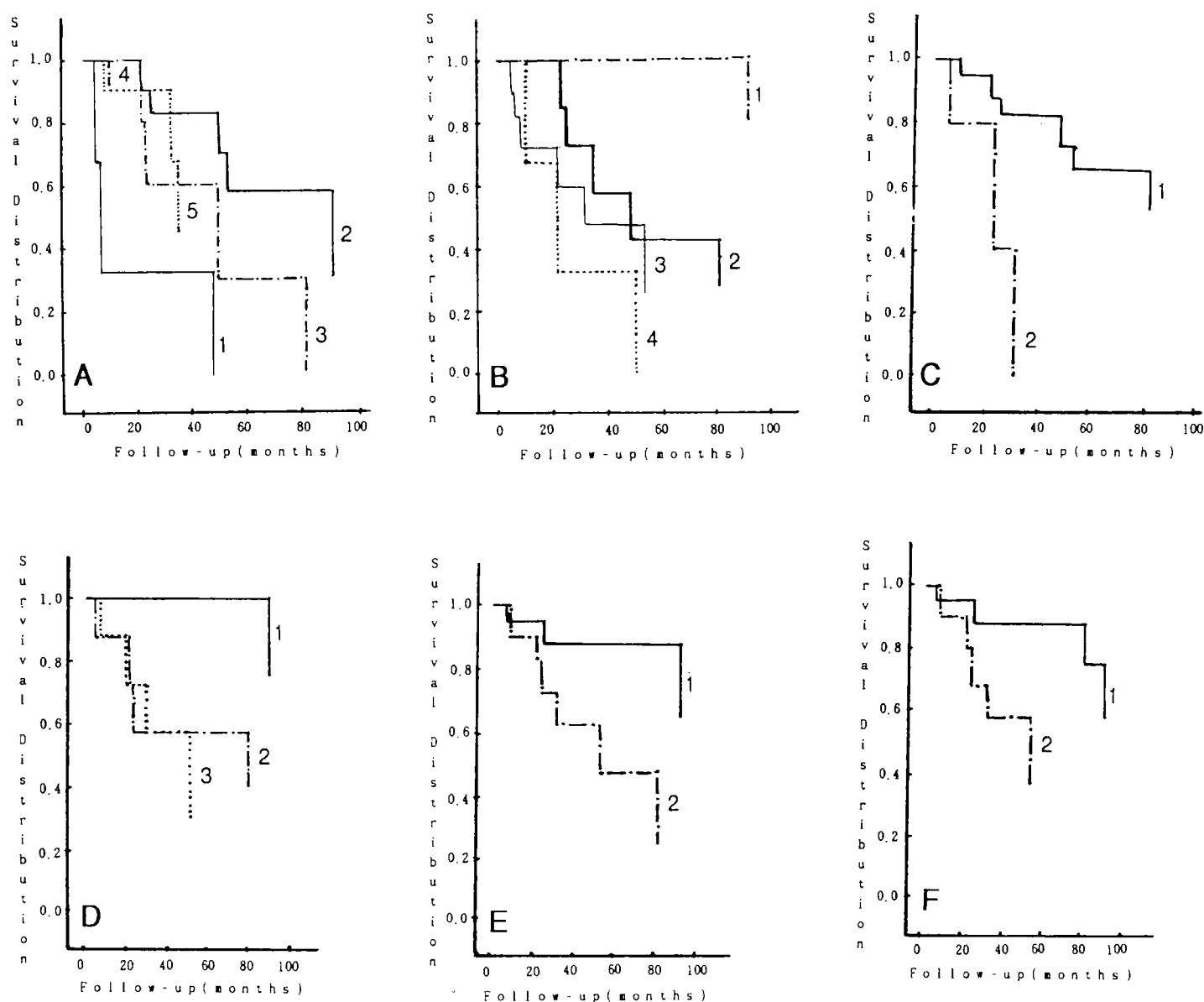


Fig. 2. Survival curves of the patient's age (A), surgical stage (B), proliferative index(PI) (C) and histologic grade (D) including differentiation of tumor cell (E) and mitotic count (F). (A) 1. less than 30 year, 2. 30-39 year, 3. 40-49 year, 4. 50-59 year, 5. more than 60 year. ($p < 0.0005$) (B) 1. stage I, 2. stage II, 3. stage III, 4. stage IV. ($p < 0.0005$) (C) 1. $PI < .55$, 2. $PI \geq 55$. ($p < 0.01$) (D) 1. grade I, 2. grade II, 3. grade III. ($p < 0.05$) (E) 1. well differentiated, 2. poorly differentiated. ($p < 0.05$) (F) 1. mitotic count $< 10/HPF$, 2. mitotic count $\geq 10/HPF$. ($p < 0.05$) HPF: high power field ($\times 400$)

Table 2. Multivariate analysis of the prognostic factors

Prognostic factors	Estimate	Standard error	p-value
Proliferative index	-1.126	0.255	<0.0005
Stage	-0.626	0.150	<0.0005
Age	0.274	0.097	<0.005
Grade	0.094	0.289	NS
Scale	0.323	0.091	

NS : not significant

counting methods, delay in the fixation of the tumor, limitation of adequate sampling of the tumor and different optical equipment were all obstacles in obtaining a reliable result. Some investigators suggested that mitotic index, i.e., the number of mitotic figures with reference to the number of nuclei, would probably be a better measure (Myhre-Jensen *et al.* 1991). Furthermore, Ueda *et al.* (1989) reported that immunohistochemical staining against Ki-67 antigen, a proliferating cell marker, would be of prognostic significance. Current study using the

antibody against PCNA showed close correlation between the patient's survival and proliferative index. Furthermore it would be an independent prognostic factor separated from other parameters such as stage and grade. The result of the staining method against PCNA is easy to read because of its distinct nuclear staining. Although delayed fixation would produce a less sensitive result, its influence might be negligible. It is certain that the proliferative index using the antibody against PCNA would be a useful prognostic factor. Nevertheless the estimate of a patient's prognosis must be made considering all prognostic parameters.

Conventionally, the histologic diagnosis, size and location of the tumor resulting in the limitation of surgery, and necrosis have been known to be prognostic factors. Although our results could not confirm their usefulness, this might be due to the relative small numbers of patients. And the modification of the parameters for the grading system was also due to the above reason. It has been known that grading of STS must be essential to predict the patient's prognosis. Among the variable criteria for grading, we accepted Trojani's grading system which provides objectivity, simplicity and reproducibility.

In conclusion, we propose a proliferative index using the antibody against PCNA as a useful prognostic factor and discussed the grading system for STS with a suggestion that Trojani's grading system would be a simple, objective and reproducible method.

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