

Myoid Differentiation and Prognosis in Adult Pleomorphic Sarcomas of the Extremity

An Analysis of 92 Cases

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BACKGROUND. The results of a recent study demonstrated an association between myoid differentiation and an adverse prognosis in adult patients with pleomorphic sarcoma, as determined by 5-year metastasis-free survival rates.

METHODS. To confirm the importance of muscle differentiation on prognosis in a well controlled clinical context, 92 samples from patients with pleomorphic sarcoma of the extremity from a single institution were immunostained with 4 monoclonal antibodies believed to be correlated with myoid differentiation: α -smooth muscle actin, muscle-specific actin, desmin, and myoglobin.

RESULTS. Forty-two cases were positive for at least 1 muscle marker and 50 cases were uniformly negative. Between the two groups, there was no significant difference in tumor size, tumor extent, or patient age found; however, histologic grade was significantly higher ($P = 0.038$) in the myoid tumors. The 5-year survival differed significantly between patients with myoid tumors (35%) and those without myoid tumors (65%) ($P = 0.0054$). Myoid differentiation remained an adverse prognostic indicator after adjusting for clinically significant factors (i.e., histologic grade, tumor size, tumor extent, and patient age) ($P = 0.01$) (hazard ratio, 2.39; 95% confidence interval, 1.24–4.63). Furthermore, there was an inverse relation found between the number of myoid markers present and survival ($P = 0.004$).

CONCLUSIONS. Myoid differentiation was found to be an independent indicator of adverse prognosis in adult patients with pleomorphic spindle cell sarcoma of the extremity. *Cancer* 2003;98:805–13. © 2003 American Cancer Society.

KEYWORDS: myogenic, myoid, sarcoma, malignant fibrous histiocytoma (MFH), immunohistochemistry, prognosis.

Adult soft tissue sarcomas are a relatively uncommon group of tumors that, when poorly differentiated, frequently are categorized as pleomorphic sarcoma, spindle cell sarcoma, or malignant fibrous histiocytoma (MFH). Although tumor grade, tumor size, extracompartmental extension, and tumor depth are recognized prognostic factors, classification by histologic type appears to have less prognostic significance. To our knowledge, few centers have sufficient numbers of cases to allow a multivariate analysis including histologic type, while the shifting taxonomy of these lesions makes an accurate interinstitutional study of the contribution of histologic type to prognosis unfeasible.

In recent years the broad category of MFH has been questioned because the majority of lesions in this category have been shown to have some differentiating features of myoid, nerve sheath, myofibroblastic, or adipose differentiation. Recently, an analysis of a group of MFH-like lesions for antigens associated with muscle differentiation

TABLE 1
Antibody Source and Concentration

Antibody	Type	Source	Concentration	Clone
Antidesmin	MoAb mouse	Dako ^a	1:40	M0724
Antimyoglobin	Rabbit	Dako ^a	1:1600	A0324
Antimuscle-specific actin, (HHF35)	MoAb mouse	Enzo ^b	1:50	C34931
Anti- α -smooth muscle actin	MoAb mouse	Sigma ^c	1:200	A2547
Anti-h-caldesmon	MoAb mouse	Dako ^a	1:50	h-CD
Antimyogenin	MoAb mouse	Dako ^a	1:100	F50

MoAb: monoclonal antibody.

^a Dako Corporation, Carpinteria, CA.

^b Enzo Diagnostics, Inc., Syosset, NY.

^c Sigma Chemical Company, St. Louis, MO.

(smooth muscle and muscle-specific actins, desmin, and myoglobin) demonstrated a negative correlation between myoid antigen expression and 5 year survival. To confirm the prognostic significance of myoid antigen expression, we analyzed a group of 92 adult pleomorphic extremity sarcoma cases from a single institution.

MATERIALS AND METHODS

Ninety-two cases with adult pleomorphic sarcomas of the extremity were identified from the University of Chicago Orthopedic Oncology database for a period extending from 1986 to 1998. The use and selection of human tumor specimens followed the guidelines approved by the Institutional Review Board of the University of Chicago. Patients with known metastases at the time of diagnosis were excluded. Cases diagnosed as embryonal or alveolar rhabdomyosarcoma, Ewing sarcoma, liposarcoma (except pleomorphic), low-grade myxoid sarcomas of any histology, epithelioid sarcoma, and synovial sarcoma were excluded. The remaining group all shared a similar phenotype of predominantly pleomorphic spindle cell histology. Histologic analysis and grading were done following published criteria.¹ Criteria for classification as leiomyosarcoma were staining for one or more of the four analyzed myoid markers, compatible histologic pattern, and positive staining for h-caldesmon. Criteria for classification as rhabdomyosarcoma were staining for desmin and/or muscle-specific actin, compatible histologic pattern, and positive staining for myogenin. Tumors were staged using the American Joint Committee on Cancer (AJCC)² and the Enneking systems.³ Tumor extent was designated as subcutaneous, deep intracompartmental, deep extracompartmental, and deep bicompartamental.

Tumor size was measured as the greatest maximum dimension of excised specimens. Because this

measurement varied according to previous treatment or prior excision, size for statistical analysis was taken from the AJCC staging with tumors categorized as ≤ 5 cm or > 5 cm.

Immunohistochemistry

Five-micron paraffin sections were deparaffinized through xylene and rehydrated through graduated alcohols to phosphate-buffered saline. Antigen retrieval was performed by microwaving in 0.1 M of citrate buffer (pH 6.0) for 10 minutes. Primary incubation was performed for 1 hour at 27 °C using the antibodies and concentrations shown in Table 1. Super Sensitive Multilink (BioGenex, San Ramon, CA) and Super Sensitive Label (BioGenex) then were applied, respectively, to each slide for 30 minutes, followed by diaminobenzidine (DAB) (Pierce, Rockford, IL) as substrate and Light Green (Fisher Scientific, Pittsburgh, PA) as counterstain. Those tumors that were consistent morphologically and immunohistochemically with pleomorphic leiomyosarcoma or pleomorphic rhabdomyosarcoma were additionally stained with h-caldesmon and myogenin. For these two antibodies, the biotin-avidin system on the Ventana automated immunostainer with the Ventana immunohistochemistry detection system (Ventana Medical Systems, Tucson, AZ) was used with DAB as a chromogen and with hematoxylin as a counterstain. A nonimmune primary antibody was substituted as the negative control. Normal tissues containing skeletal and smooth muscle served as positive controls. Stained samples were evaluated independently by two investigators (A. G. M. and A. T. D.).

Statistical Analysis

All statistical analyses were performed using STATA, release 7 (Stata Corporation, College Station, TX). The Fisher exact test was used to compare categorical demographic, clinical, and pathologic characteristics be-

TABLE 2
Summary of Clinicopathologic Data, Prognostic Characteristics, and Myoid Immunohistochemical Analyses of the 92 Adult Pleomorphic Sarcomas in the Current Study

Prognostic factors	Total (n = 92) No. (%)	Muscle differentiation		P-value ^a
		Yes (n = 42) No. (%)	No (n = 50) No. (%)	
Gender				
Female	48 (52.2)	20 (47.6)	28 (56.0)	0.53
Male	44 (47.8)	22 (52.4)	22 (44.0)	
Histologic grade (from AJCC)				
1	11 (12.0)	5 (11.9)	6 (12.0)	0.038
2	7 (7.6)	0 (0.0)	7 (14.0)	
3	74 (80.4)	37 (88.1)	37 (74.0)	
Grade (from Enneking system)				
Low	12 (13.0)	5 (11.9)	7 (14.0)	1.00
High	80 (87.0)	37 (88.1)	43 (86.0)	
Size (from AJCC)				
≤ 5 cm	22 (23.9)	9 (21.4)	13 (26.0)	0.63
> 5 cm	70 (76.1)	33 (78.6)	37 (74.0)	
Site (from Enneking system)				
Intracompartmental	58 (63.0)	27 (64.3)	31 (62.0)	0.83
Extracompartmental	34 (37.0)	15 (35.7)	19 (38.0)	
Extent				
Subcutaneous	16 (17.4)	5 (11.9)	11 (22.0)	0.21
Deep intracompartmental	42 (45.7)	22 (52.4)	20 (40.0)	
Deep extracompartmental	25 (27.2)	9 (21.4)	16 (32.0)	
Deep bicompartamental	9 (9.8)	6 (14.3)	3 (6.0)	
Size (from pathologic measure)				
Mean (SD)	9.2 (6.0)	9.5 (6.2)	8.9 (5.9)	0.65 ^b
Range	2–26	2–26	2–26	
Age (yrs)				
Mean (SD)	56.8 (17.2)	58.1 (18.4)	55.8 (16.4)	0.52 ^b
Range	21–89	21–89	28–88	

AJCC: American Joint Committee on Cancer; SD: standard deviation.

^a Fisher exact test used, except where otherwise specified.

^b Student *t* test used.

tween patients with and those without at least one myoid marker. For continuous variables such as age, the two-sample Student *t* test was used. Survival curves were generated using the Kaplan–Meier method.⁴ The log-rank test was employed for comparing survival rates in different groups, and the Cox proportional hazards regression model was used for assessing and controlling for the effects of potential prognostic factors on survival.⁵ Proportional hazards assumption was tested by regressing Schoenfeld partial residuals on failure time.⁶ Survival analyses also were performed in patients with histologic Grade 3 tumors (based on the AJCC staging system) only to examine the effect of myoid differentiation in this subgroup. Similar analyses were conducted after excluding patients with leiomyosarcoma or rhabdomyosarcoma. A *P* value < 0.05 was considered statistically significant.

RESULTS

The patient list included 48 women and 44 men. The average age of the patients at the time of diagnosis was 56.8 years (range, 21–89 years) (Table 2). Follow-up was obtained for all patients and the median follow-up time for survivors was 64.8 months (range, 21.4–169.1 months). The original diagnoses included leiomyosarcoma (8 patients), fibrosarcoma (8 patients), pleomorphic liposarcoma (4 patients), round cell liposarcoma (1 patient), malignant peripheral nerve sheath tumor (7 patients), synovial sarcoma (1 patient), MFH (48 patients), and spindle cell sarcoma, not otherwise specified (NOS) (15 patients).

The majority of the patients had high-stage tumors according to the AJCC staging system (4 cases of Stage IA, 7 cases of Stage IB, 3 cases of Stage IIA, 4 cases of Stage IIB, 15 cases of Stage IIIA, and 59 cases of Stage IIIB disease) and the Enneking system (6 cases

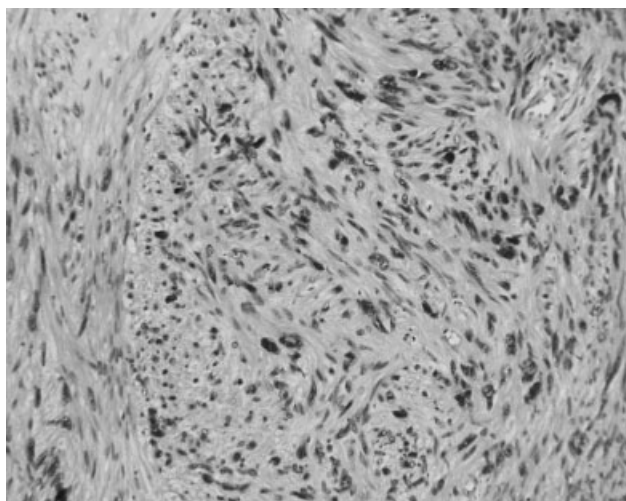


FIGURE 1. Representative histologic appearance of adult pleomorphic sarcoma (H & E). The tumors were comprised primarily of spindle cells with moderate to severe nuclear pleomorphism. Cases diagnosed as embryonal or alveolar rhabdomyosarcoma, Ewing sarcoma, liposarcoma (except pleomorphic), low-grade myxoid sarcomas of any histology, epithelioid sarcoma, and synovial sarcoma were excluded.

of Stage IA, 6 cases of Stage IB, 52 cases of Stage IIA, and 28 cases of Stage IIB disease). There were 16 superficial and 76 deep tumors; 58 tumors were intracompartmental and 34 were extracompartmental.

Histologic and Immunohistochemical Analysis

All tumors had a predominant pleomorphic spindle cell histologic pattern (Fig. 1). Among all tumors, 42 cases were positive for at least 1 myoid marker, including 10 tumors that were positive for desmin (11%), 17 tumors that were positive for muscle-specific actin (18%), 38 tumors that were positive for α -smooth muscle actin (41%), and 7 tumors that were positive for myoglobin (8%) (Fig. 2). Twenty tumors (22%) were positive for a single marker, 14 tumors (15%) were positive for 2 markers, 8 tumors (9%) were positive for 3 markers, and 50 tumors (54%) were negative for all markers. No tumors were found to be positive for all four markers. In cases in which 2 markers were positive, 9 cases (10%) were positive for muscle-specific actin and α -smooth muscle actin, 3 cases were positive for α -smooth muscle actin and desmin, and 2 cases were positive for α -smooth muscle actin and myoglobin. In cases in which three markers were positive, four cases were positive for desmin, muscle-specific actin, and α -smooth muscle actin and four cases were positive for muscle-specific actin, α -smooth muscle actin, and myoglobin.

After a review of the histology and immunohisto-

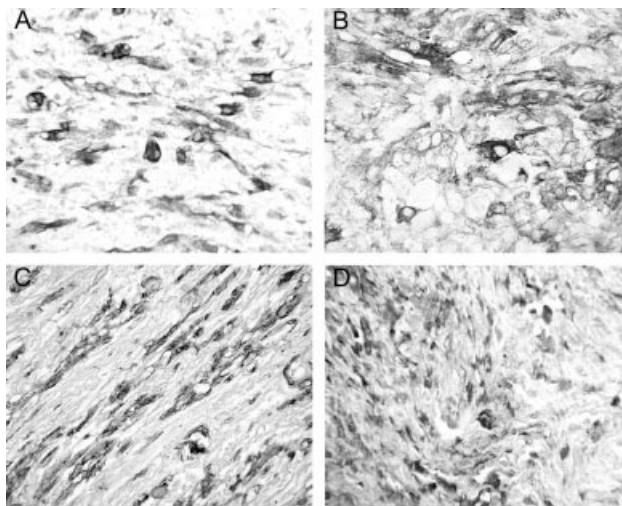


FIGURE 2. Immunohistochemical analysis of myogenic marker expression in adult pleomorphic sarcomas of the extremity. Each selected tumor was accompanied by hematoxylin and eosin staining to verify tumor histology. Tumor sections were incubated with four antibodies to proteins associated with myoid differentiation: desmin, α -smooth muscle actin, muscle-specific actin, and myoglobin. The presence of antibody was visualized using the HRP-diaminobenzidine detection system, followed by Light Green counterstaining. Four representative cases are shown: (A) positive desmin staining; (B) positive α -smooth muscle actin staining; (C) positive muscle-specific actin staining; and (D) positive myoglobin staining (magnification $\times 200$).

chemical analysis, 11 tumors that had a histologic appearance consistent with leiomyosarcoma (eight tumors) or rhabdomyosarcoma (three tumors) as well as staining for myoid markers were evaluated further for h-caldesmon and myogenin positivity, respectively. None of the putative rhabdomyosarcomas was found to be positive for myogenin and two of the putative leiomyosarcomas were negative for h-caldesmon. Consequently, a total of six cases were classified as leiomyosarcoma and no cases satisfied the criteria for rhabdomyosarcoma. Histologic reevaluation of the remaining 36 cases with myoid antigen reactivity revealed 14 pleomorphic myogenic sarcomas, 20 myofibroblastic sarcomas, and 2 malignant peripheral nerve sheath tumors with myoid antigens. Reevaluation of the 50 cases that were negative for myoid markers revealed 10 cases of pleomorphic liposarcomas; 20 cases of MFH; 11 cases of pleomorphic sarcomas, NOS; 5 malignant peripheral nerve sheath tumor cases; 3 myxofibrosarcoma cases; and 1 fibrosarcoma case.

Cases were divided into those with (42 cases) and those without (50 cases) at least 1 myoid marker. Comparison of these groups for clinical factors considered to influence survival showed no difference with regard to tumor size, depth, or stage or with

TABLE 3
Correlation between Myoid Immunohistochemical Staining and Patient Survival

	No. of deaths	1-year survival (%)	2-year survival (%)	5-year survival (%)	Log-rank test, <i>P</i> value
Myoid differentiation					
No (<i>n</i> = 50)	17	98.0	86.0	64.9	0.0054
Yes (<i>n</i> = 42)	27	85.7	73.8	35.3	

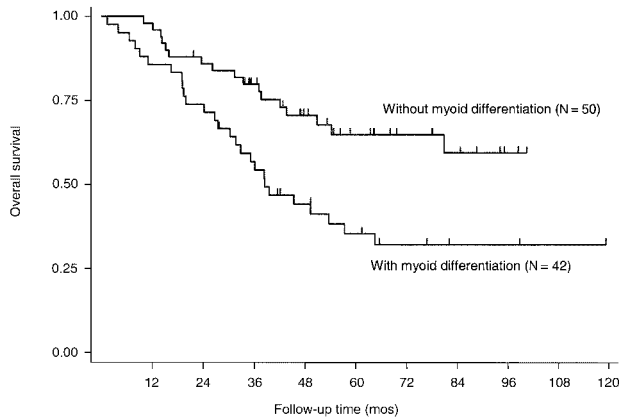


FIGURE 3. Kaplan–Meier survival curve for patients with and without myogenic differentiation. The figure demonstrates biostatistical correlation between the expression of at least one myoid marker and survival in patients with adult pleomorphic sarcoma of the extremity. Of 92 patients, 44 died during the study. In the myoid marker-positive group, the median survival time was 38.4 months (95% confidence interval, 30.3–64.5 months). In the myoid marker-negative group, the median survival time was not achieved after a median of 64.8 months of follow-up (range, 21.4–169.1 months).

regard to the age or gender of the patient at the time of diagnosis (Table 2). Histologic grade (AJCC) was found to be significantly higher ($P = 0.038$) in those tumors that were positive for myoid markers.

Survival Analyses

There was a significant difference in the 5-year survival rate between patients with (35%) and those without (65%) myoid differentiation ($P = 0.0054$ by the log-rank test) (Table 3) (Fig. 3). The unadjusted hazard ratio for myoid differentiation, as defined by at least 1 positive marker, was 2.31 (95% confidence interval [95% CI], 1.26–4.25). After adjusting for clinically significant factors including histologic grade (AJCC), tumor size, tumor extent, and patient age, myoid differentiation remained a statistically significant adverse prognostic indicator with an adjusted hazard ratio of 2.39 (95% CI, 1.24–4.63) (Table 4). Adjustment for a 2-tiered histologic grading system (Enneking system)

TABLE 4
Multivariate Cox Models with Dichotomous Variable of Myoid Differentiation

	Hazard ratio	95% CI	<i>P</i> value
Myoid differentiation	2.39	1.24–4.63	0.01
Grade (AJCC) 2 vs. 1	0.35	0.04–3.34	0.36
Grade (AJCC) 3 vs. 1	0.84	0.32–2.26	0.74
Size (AJCC) > 5 vs. ≤ 5 cm	5.32	1.73–16.33	0.003
Deep compartmental vs. subcutaneous	1.04	0.38–2.85	0.94
Deep intracompartmental vs. subcutaneous	1.42	0.49–4.14	0.52
Deep bicompartamental vs. subcutaneous	3.51	0.96–12.92	0.058
Age in 10-yr increments	1.27	1.04–1.56	0.021

95% CI: 95% confidence interval; AJCC: American Joint Committee on Cancer.

TABLE 5
Correlation between Myoid Immunohistochemical Staining and Patient Survival

	No. of deaths	1-year survival (%)	2-year survival (%)	5-year survival (%)	Log-rank test, <i>P</i> value
Myoid markers					
0 (<i>n</i> = 50)	17	98.0	86.0	64.9	0.005
1 (<i>n</i> = 20)	12	90.0	80.0	36.9	
2 (<i>n</i> = 14)	10	92.9	78.6	33.3	
3 (<i>n</i> = 8)	5	62.5	50.0	37.5	

gave similar results (hazard ratio of 2.64; 95% CI, 1.40–5.00) ($P = 0.003$). Because of the significant difference in histologic grade between those patients with and those without myoid differentiation, a comparison of survival was undertaken for only those patients with histologic Grade 3 tumors ($n = 74$). Of these, 37 tumors were positive for at least 1 muscle marker and 37 tumors were negative. The adjusted hazard ratio for myoid differentiation was 2.04 (95% CI, 1.01–4.12) after adjusting for tumor size, tumor extent, and patient age. These data confirm that the poorer prognosis in myoid sarcoma patients is not simply attributable to a higher grade histology in that subset. After 6 patients with confirmed leiomyosarcoma were excluded, the adjusted hazard ratio for myoid differentiation was 2.18 (95% CI, 1.08–4.40) after controlling for histologic grade (AJCC), tumor size, tumor extent, and patient age.

To assess whether positivity for myoid markers was additive, survival analysis for patients was calculated according to the number of positive markers (Table 5) (Fig. 4). Compared with patients with negative myoid markers, the unadjusted hazard ratios for patients with 1, 2, and 3 positive myoid markers were 2.10 (95% CI, 1.00–4.40), 2.34 (95% CI, 1.07–5.10), and

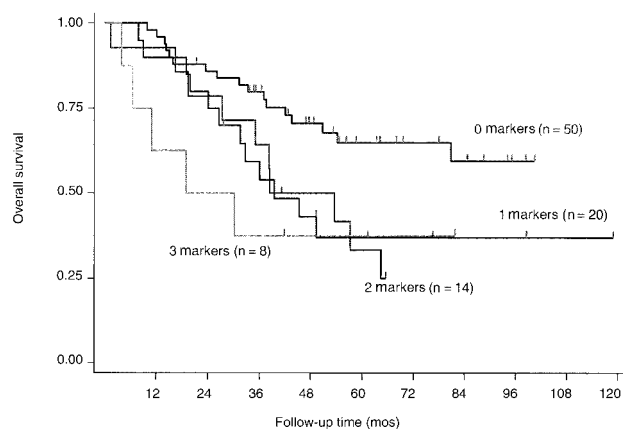


FIGURE 4. Kaplan–Meier survival curve for patients with immunohistochemical positivity for zero, one, two, and three myoid markers. The figure demonstrates biostatistical correlation between the expression of increasing numbers of myoid markers and survival in patients with adult pleomorphic sarcoma of the extremity. Of 92 patients, 20 tumors (22%) were positive for a single marker, 14 tumors (15%) were positive for 2 markers, 8 tumors (9%) were positive for 3 markers, and 50 tumors (54%) were negative for all markers. Compared with patients who were myoid marker-negative, the unadjusted hazard ratios for patients with 1, 2, and 3 positive myoid markers were 2.10 (95% confidence interval [95% CI], 1.00–4.40), 2.34 (95% CI, 1.07–5.10), and 2.99 (95% CI, 1.10–8.12), respectively, demonstrating that increasing myoid differentiation is correlated with worse survival.

2.99 (95% CI, 1.10–8.12), respectively, demonstrating that increasing myoid differentiation is correlated with worse survival. After adjustment for histologic grade (AJCC), tumor size, tumor extent, and patient age, the hazard ratios still increased as the number of positive myoid markers increased, although the increase was not monotonic because of small sample size (Table 6). The adjusted hazard ratio per score increased was 1.57 (95% CI, 1.15–2.13; $P = 0.004$), which indicates a 57% increase in the risk of death for each additional positive myoid marker. In the subgroup of patients with histologic Grade 3 disease ($n = 74$), the adjusted hazard ratio per score increment was 1.50 (95% CI, 1.06–2.11; $P = 0.021$). Finally, the adjusted hazard ratio per score increment was 1.57 (95% CI, 1.09–2.26; $P = 0.015$) after excluding patients with leiomyosarcoma ($n = 86$).

DISCUSSION

Pleomorphic sarcomas have undergone 2 taxonomic revolutions in the past 30 years. The identification of MFH as a diagnostic category was followed by a period of increasing popularity for this diagnosis, leading to a sharp decrease in the diagnosis of more traditional pleomorphic sarcomas such as rhabdomyosarcoma, fibrosarcoma, and liposarcoma.^{7–10} With the develop-

TABLE 6
Multivariable Cox Model with Ordinal Variable of Muscle Differentiation

	Hazard ratio	95% CI	P value
Muscle diff score 1 vs. 0	1.82	0.82–4.01	0.14
Muscle diff score 2 vs. 0	3.25	1.42–7.43	0.005
Muscle diff score 3 vs. 0	2.91	0.92–9.18	0.068
Grade (AJCC) 2 vs. 1	0.37	0.04–3.71	0.40
Grade (AJCC) 3 vs. 1	0.95	0.33–2.71	0.92
Size (AJCC) > 5 vs. ≤ 5 cm	5.73	1.82–18.02	0.003
Deep compartmental vs. subcutaneous	1.11	0.39–3.19	0.84
Deep intracompartmental vs. subcutaneous	1.43	0.46–4.49	0.84
Deep bicompartamental vs. subcutaneous	4.04	1.05–15.59	0.043
Age in 10-yr increments	1.28	1.04–1.58	0.02

95% CI: 95% confidence interval; muscle diff: muscle differentiation; AJCC: American Joint Commission on Cancer.

ment of more sophisticated ancillary studies, the category of MFH has been recognized as encompassing a heterogeneous group of tumors sharing a common phenotype, but frequently expressing features that could be classified more specifically with the use of immunohistochemistry, electron microscopy, or molecular studies. Close reexamination of older series of MFH-like tumors generally allows reclassification of greater than half of those cases into a more specific diagnostic category.^{11,12} As a consequence, entities such as pleomorphic rhabdomyosarcoma and liposarcoma are reportedly increasing in incidence and, depending on the philosophy of the diagnostic center, MFH is becoming a less commonly diagnosed entity.⁸

Many institutions use the generic terms “MFH-like” or “pleomorphic sarcoma” in lieu of conducting an extensive and expensive diagnostic workup to delineate a specific histologic type. This practice is justified by the belief that once small blue cell tumors, synovial sarcoma, angiosarcoma, myxoid sarcomas, and other dissimilar entities are excluded, further histologic classification confers no additional information beyond that provided by conventional staging and prognostic factors such as tumor grade, size, depth, and confinement to a compartment. Indeed, because all pleomorphic sarcomas tend to be treated very similarly, the treatment algorithm becomes similar to that of lung carcinomas, which are viewed as either “small cell” or “non-small cell” in terms of management.

There are inherent difficulties in demonstrating the independent significance of histologic type in pleomorphic sarcomas. They are uncommon tumors, and to our knowledge only a few centers have accrued sufficient cases to date to allow multivariate analysis.

The majority of studies include pleomorphic sarcomas, blue cell tumors, myxoid tumors, tumors of unknown histogenesis, and even sarcomas from internal organs. Hashimoto et al.,¹³ in reviewing 1116 soft tissue sarcomas, included retroperitoneal and urogenital sites as well as small blue cell tumors and found that grade and tumor necrosis but not histologic type were correlated inversely with survival. In a study of 1240 sarcoma patients subjected to a multivariate Cox model, Coindre et al.¹⁴ reported no significant effect of histologic type on survival when controlled for tumor size, grade, and depth. That study included all soft tissue sarcomas of any type, including small blue cell tumors and others of uncertain histogenesis, as well as tumors of the retroperitoneum. A multivariate analysis of 1041 localized soft tissue sarcomas of the extremities from a group at Memorial Sloan-Kettering¹⁵ found that disease-specific survival was affected adversely by tumor size, grade, and location, and the histologic types *leiomyosarcoma* and *malignant peripheral nerve sheath tumor*. When the case selection is narrowed to similar site and cell type, histologic classification begins to emerge as an independent prognostic factor.

The current study was prompted by the report of Fletcher et al.¹² in which the presence of muscle markers in a group of 100 MFH-like tumors was found to be an independent negative prognostic factor. That study found 30 cases with myoid differentiation from an initial pool of 100 cases, and in a univariate analysis demonstrated an adverse effect on survival ($P < 0.006$). Unfortunately, the myoid tumors as a group were more frequently deep ($P < 0.04$) and of high grade ($P < 0.02$), both factors that are known to affect survival and were not controlled for in the univariate analysis. In the current study there was no significant difference between the myoid and nonmyoid groups with regard to tumor size, tumor depth, compartmental extent, patient age, or patient gender. There was a significant difference with regard to grade on a three-grade scale ($P = 0.038$), which was absent when grading was performed using the two-grade Enneking scale. This bias was compensated for on the multivariate analysis, which showed myoid differentiation to still be significant. In addition, analysis of high-grade tumors only ($n = 74$) showed myoid differentiation to still have significance. More important, prognosis is affected by the presence of any of the myoid markers, and shows an additive effect.

Myoid differentiation is observed in a broad group of tumors representing tumors with skeletal or smooth muscle differentiation including rhabdomyosarcoma, leiomyosarcoma, and myoepithelial tumors, as well as tumors of myofibroblastic origin. Unfortunately, the expression of myoid antigens is not limited to these

categories and cannot be taken, a priori, as evidence of smooth muscle differentiation. Muscle-specific actin has been demonstrated in phyllodes tumor of the breast,¹⁶ melanoma,¹⁷ and in 21% of monophasic synovial sarcomas, whereas myoglobin has been reported in peripheral neuroectodermal tumors,¹⁸ alveolar soft part sarcoma, and granular cell tumor. Pleomorphic liposarcomas have been reported to express smooth muscle actin and desmin in 49% and 19% of cases, respectively.¹⁹ In a review of cytogenetically confirmed cases of myxoid liposarcoma, five of nine cases had desmin expression, two of which also expressed both muscle-specific and smooth muscle actins.²⁰ Expression of a single myoid antigen alone is not sufficient to consider a tumor to be of skeletal or smooth muscle differentiation.

Conversely, myoid tumors may lose the expression of muscle antigens. Oda et al. found that pleomorphic leiomyosarcoma expresses reactivity for smooth muscle actin, muscle-specific actin, and desmin in only 50%, 46%, and 36% of cases, respectively.²¹ Four of 14 well characterized pleomorphic rhabdomyosarcoma cases reported by Schurch et al.⁸ were negative for desmin, whereas 4 desmin-positive cases also were found to be positive for smooth muscle actin. A review of 38 cases of pleomorphic rhabdomyosarcoma at the Armed Forces Institute of Pathology²² revealed 37 of 38 cases studied were positive for myoglobin, but only 19 of 36 cases and 19 of 34 cases expressed MyoD1 and myogenin, respectively, both of which are markers of early skeletal muscle differentiation, whereas 15 of 35 cases expressed smooth muscle actin. The absence of any one characteristic marker is not sufficient to exclude the diagnosis of a tumor with myogenic differentiation.

The classification of tumors expressing muscle markers is an exercise requiring the careful integration of histologic appearance and special studies. When multiple muscle markers are positive in the presence of a compatible histology, the diagnosis of leiomyosarcoma or rhabdomyosarcoma can be made with reasonable certainty. However, none of the screening myoid markers used in the current study can be considered to be definitive evidence of smooth or skeletal muscle differentiation in isolation. The classification of pleomorphic sarcomas is still evolving, with variability between institutions. Indeed, although small round cell sarcomas, liposarcoma, and synovial sarcoma have a high interobserver reproducibility, the classification of pleomorphic sarcomas is reported to be much less reproducible.²³ For this reason, these tumors may be considered empirically as pleomorphic sarcomas with myoid antigen expression, without further classification.

What is the biologic basis for the strong correlation of myoid antigen expression with poor survival? One possibility is that the myoid antigen-positive group includes a subset with a known poorer prognosis (i.e., leiomyosarcoma and rhabdomyosarcoma). After immunohistochemical and histologic reevaluation, six of the myoid antigen-positive cases were classified as pleomorphic leiomyosarcoma, and both pleomorphic leiomyosarcoma and rhabdomyosarcoma have been reported previously to have a poor prognosis. Pisters et al. found in an analysis of 1041 extremity sarcomas that leiomyosarcoma was a poor prognostic factor for disease-specific survival.¹⁵ Singer et al. found that MFH, leiomyosarcoma, and pleomorphic rhabdomyosarcoma had a significantly worse survival than liposarcoma and fibrosarcoma.²⁴ In the latter study, there was no attempt to recategorize lesions diagnosed as MFH, and it is likely that some of the lesions in that category would correspond to the pleomorphic sarcomas with myoid antigen expression reported in the current study. Although at least part of the negative effect on survival can be attributed to the six cases of pleomorphic leiomyosarcoma, the effect remained significant even when these cases were excluded from analysis. The presence of even a single myoid marker was shown in the current study to have a significant negative impact on survival.

Deviant expression of actin and desmin may be an epiphenomenon of poor or divergent differentiation, and hence a marker of poor prognosis in a broad category of pleomorphic or MFH-like lesions. This model is observed in other tumors, such as the independent adverse effect on survival reported in carcinomas of the breast that express the β subunit of human chorionic gonadotropin.²⁵ The dosage effect of increasing myoid marker expression observed in the current study may reflect increasingly aberrant differentiation. A similar effect on survival was reported by the Chromosomes and Morphology (CHAMP) study group, which identified five cytogenetic abnormalities in soft tissue sarcomas that were reported to be independent predictors of adverse outcome, and demonstrated a similar dosage effect.²⁶ Alternatively, pleomorphic sarcomas with myoid antigen expression may encompass one or more separate biologic entities represented by the diagnostic categories of myofibroblastic sarcoma, pleomorphic myoid sarcoma, and others as described by Fletcher et al.¹²

The demonstration of myoid antigens in adult pleomorphic soft tissue sarcoma of the extremity was found on multivariate analysis to have a negative impact on survival that is independent of other prognostic factors, a finding that confirms the previous observation of Fletcher et al.¹² Regardless of views

concerning the classification of these lesions, efforts should be made to identify those pleomorphic sarcomas that express myoid antigens because patients with these tumors may benefit from the development and use of better adjuvant therapies.

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