Extraskeletal Myxoid Chondrosarcoma: A Clinicopathologic, Immunohistochemical, and Ploidy Analysis of 23 Cases

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Twenty-three cases of extraskeletal myxoid chondrosarcoma, evaluated at the Mayo Clinic between 1968 and 1996, were studied for clinicopathologic features, immunohistochemical profile, Ki-67 activity, and ploidy status to identify adverse prognostic factors. Females and males were equally affected, and the median age at diagnosis was 50 years. The tumors were located mainly in the lower extremities (83%), and the median tumor size was 9.5 cm. Sixteen tumors showed low cellularity (70%), and eight tumors had high mitotic activity (more than two per 10 high-power fields). The tumors were immunoreactive for vimentin (89%), synaptophysin (72%), epithelial membrane antigen (28%), and S-100 protein (17%). Nine tumors were diploid, three aneuploid, and one tetraploid. Mean Ki-67 activity was 11% (range, 1 to 45%). The 10-year overall survival rate was 78%. On univariate analysis, tumor size ≥ 10 cm, high cellularity, presence of anaplasia or rhabdoid features, mitotic activity more than two per 10 high-power fields, Ki-67 ≥ 10%, and Ki-67 "hot spot" ≥ 25% were associated with decreased metastasis-free or overall survival. Ploidy status was not associated with any adverse outcome. The presence of any of these adverse prognostic factors can indicate the possibility of a more aggressive behavior in extraskeletal myxoid chondrosarcoma, and a closer follow-up is suggested.

KEY WORDS: Extraskeletal myxoid chondrosarcoma, Immunohistochemistry, Ki-67, Ploidy, Prognostic factors.

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Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue sarcoma that was recognized as a distinct pathologic entity by Stout and Verner in 1953 (1). However, it was not until 1972 that Enzinger and Shiraki (2) defined the clinicopathologic features of EMC, showing a relatively protracted clinical course and a better prognosis than that with conventional bone chondrosarcomas.

In 1992, Saleh *et al.* (3) reassessed the clinical features of EMC in a series of 10 patients and showed the "indolent but resilient" nature of this neoplasm; 7 of the patients died of tumor up to 17 years after the initial diagnosis. Seven years later, Meis-Kindblom *et al.* (4) studied a large series of EMCs, most consultation cases, and showed that older age, larger tumor size, and proximal tumor location were associated with decreased survival by multivariate analysis.

The histogenesis of EMC is still a subject of controversy. However, chondroblastic differentiation has been supported by ultrastructural (5–15) and histochemical (16–18) studies. In addition, cytogenetic and molecular analyses have shown that EMC is a distinct entity with the characteristic translocation t(9;22) involving the *EWS* gene (22q12) and the *TEC* gene (9q22) in a majority of the cases (19–29).

We reviewed the clinicopathologic, immunohistochemical, and ploidy features in a series of 23 cases of EMC evaluated at a single institution to identify potential prognostic factors associated with a more aggressive behavior.

MATERIALS AND METHODS

Twenty-three patients in whom EMC was diagnosed between 1968 and 1996 were included in this study. Eighteen patients with EMC were diagnosed and primarily treated at the Mayo Clinic, and five patients were referred by other institutions for additional treatment or evaluation. Fifteen cases from this series will be described in another article (30).

TABLE 1. Antibodies Used in This Study

Antibody	Type	Source	Dilution
Vimentin 3B4	M	DAKO	1:500
Desmin Der11	M	DAKO	1:100
Wide-spectrum keratin	P	DAKO	1:200
Epithelial membrane antigen (EMA)	M	DAKO	1:20
Polyclonal carcinoembryonic antigen (pCEA)	P	DAKO	1:800
S-100 protein	P	HSC	1:2,000
Synaptophysin SY38	M	ICN Biomedicals	1:40
Chromogranin	M	Boehringer Mannheim	1:1,000
Leu-7 (CD57)	M	Becton Dickinson	1:20
Actin HHF35 (actin muscle-specific)	M	DAKO	1:50
α-Smooth muscle actin 1A4	M	DAKO	1:150
MIC2 (CD99)	M	DAKO	1:50
Glial fibrillary acid protein (GFAP)	P	DAKO	1:300
Ki-67 (MIB-1)	M	Immunotech	1:50

HSC, Hospital for Sick Children; M, monoclonal; P, polyclonal.

Clinical data, including follow-up information, were obtained from medical record review, referring physicians, and telephone survey.

Hematoxylin-eosin–stained archival slides from the primary tumors (5 to 21 slides per case; average, 13) were available for review in all cases. The main histologic features investigated were cellularity, mitotic activity, anaplasia, spontaneous necrosis, and vascular invasion. Cellularity was defined as high when the neoplastic cells represented 75% or more (and the myxoid matrix represented 25% or less) of the tumor area within the nodules in at least 25% of all slides evaluated per tumor. Mitotic count was performed in 10 different high-power fields (HPF, 400×) and reported as the number of mitotic figures per 10 HPF. Anaplasia was defined as the presence of enlarged and vesicular nuclei with prominent nucleoli in at least 25% of the tumor area.

Immunohistochemical studies were performed on 4-μm-thick sections of 10% formalin-fixed, paraffin-embedded tumor material in 18 tumors, according to the avidin-biotin-peroxidase complex system (31). Appropriate positive and negative controls were used for each antibody listed in Table 1. Immunohistochemical analysis addressed the presence or absence of stain in a focal or diffuse pattern. Focal reactivity was defined when less than 25% of cells were reactive for a specific antibody. Immunoreactivity intensity was not categorized.

Digital image analysis was performed in 13 neoplasms according to a previously described

TABLE 2. Clinical Features of Extraskeletal Myxoid Chondrosarcoma in 23 Patients

Case	Age (yr)	Sex	Location	Size (cm)*	Cellularity	Ploidy	Treatment	Follow-up and Outcome
1	47	M	Thigh, left	16	Low	D	WLE + postRx	Lung, sternum, vertebral mets (10 mo); DOD (23 mo)
2	29	M	Thigh, left	18	Low	D	WLE + postRx	Lung mets (9 yr), DOD (22 yr)
3	34	M	Mets to chest wall, mediastinum, and lungs (primary tumor: knee, right)	4†	Low	D	ME + postRx	Knee tumor discovered (4.3 yr), alive (13 yr)
4	57	F	Ankle, right	1.5	Low	D	WLE	Alive, NED (21 yr)
5	27	M	Ankle, left	10	High	···	ME	Alive, NED (6 mo)
6	63	F	Thigh, right with mets to regional lymph nodes and buttock	4.5	Low	···	WLE + postRx	Alive with mets (1 yr)
7	63	F	Thigh, left	6	Low	···	WLE	Local recurrence (8 yr), alive with NED (20.3 yr)
8	42	F	Thigh, right	9	Low	···	WLE + postRx	Alive, NED (23.5 yr)
9	55	M	Thigh, left	10	High	A	WLE	Lung mets (4 mo), DOD (8 mo)
10	54	M	Thigh, right	10	High	T	PreRx + WLE	Rib and vertebral mets (3.3 yr), DOD (6 yr)
11	50	F	Shoulder, right	13.5	High	D	WLE + postRx	Lung mets (5.5 yr), DOD (6.8 yr)
12	22	F	Shoulder, right	5	High	···	WLE	Alive, NED (14.6 yr)
13	49	F	Leg, left	10	Low	···	WLE	Lung mets (1.2 yr), alive with mets (6.5 yr)
14	47	F	Ankle, right	9	High	A	Amputation	Alive, NED (8.2 yr)
15	38	F	Chest wall with lung mets	5	High	D	PreChx + WLE	Alive with mets (7 yr)
16	52	M	Thigh, right	11	Low	···	WLE + postRx	Lung mets (4 yr), alive with mets (6.8 yr)
17	78	F	Foot, right	5	Low	D	Amputation	Alive, NED (5.5 yr)
18	48	M	Foot, left	9	Low	D	Amputation	Alive, NED (6 yr)
19	70	M	Paravertebral area, left	5.5	Low	A	WLE + postRx	Alive, NED (6.2 yr)
20	71	M	Thigh, left	18.5	Low	···	PreRx + WLE + postRx	Local recurrence (3 mo), lung mets (6 mo), alive with mets (3.3 yr)
21	61	F	Knee, right	11	Low	D	WLE	Local recurrence (4.8 yr), alive with NED (17 yr)
22	44	F	Ankle, right	4	Low	···	WLE	Alive, NED (13 yr)
23	51	M	Foot, left	6.5	Low	···	Amputation	Alive, NED (2 yr)

A, aneuploid; D, diploid; ME, marginal excision; mets, metastasis; postRx, postoperative radiotherapy; preChx, preoperative chemotherapy; preRx, preoperative radiotherapy; T, tetraploid; WLE, wide local excision.

* Greatest tumor dimension.

† Knee tumor.

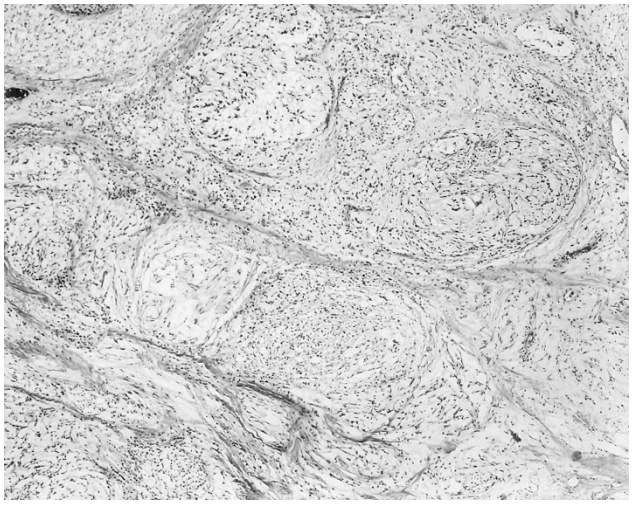

FIGURE 1. Characteristic multilobular architecture of extraskeletal myxoid chondrosarcoma. This was present in all cases.

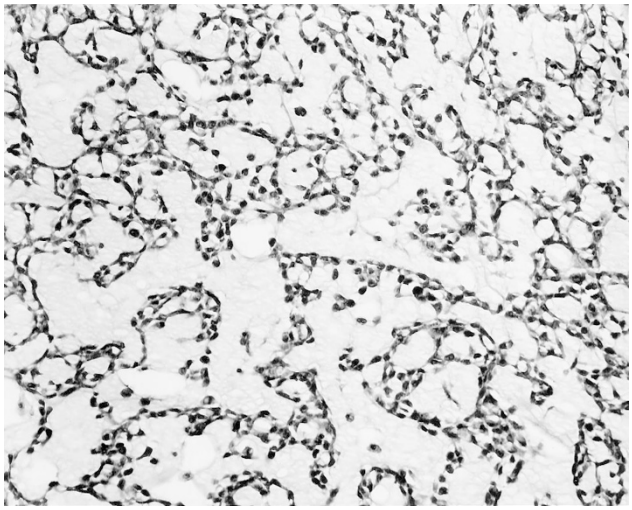

FIGURE 2. Cords and strands of small eosinophilic cells immersed in a myxoid matrix. This is the classic histologic finding in extraskeletal myxoid chondrosarcoma.

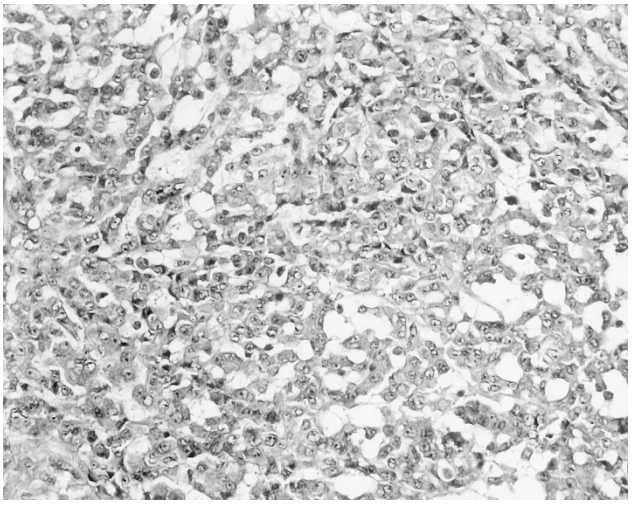

FIGURE 3. Areas composed of large cells with vesicular nuclei and prominent nucleoli were found in three tumors. Interestingly, two of these patients died of disease.

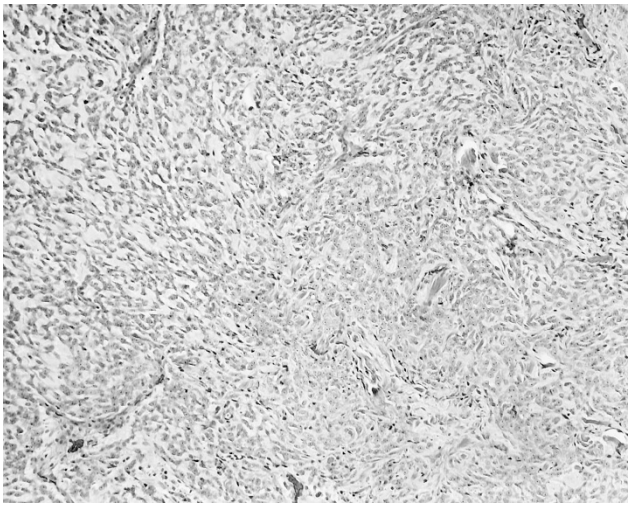

FIGURE 4. High cellularity was present in 30% of cases and was associated with decreased overall survival by univariate analysis.

method (32). An average of 158 cells were counted per tumor (range, 53 to 213 cells). Feulgen stain was used to evaluate tumor ploidy: a DNA index between 0.9 and 1.1 was considered diploid; a DNA index between 1.8 and 2.2 in more than 10% of the cells was considered tetraploid; and a DNA index between 1.11 and 1.79 or more than 2.2 was considered aneuploid. Immunoperoxidase stain for Ki-67 was used to assess the overall proliferative activity (Table 1). Ki-67 "hot spot" was arbitrarily defined as an area with 25 mm^2 around the point with the highest concentration of cells immunoreactive for Ki-67 antigen in the tumor. The total number of cells in this area was counted by digital image analysis, and the percentage of cells immunoreactive for Ki-67 was estimated. This analysis considered that malignant neoplasms are composed of a heterogeneous population of cells with distinct biological properties (33, 34), and this concept has been used in the assessment of microvessel density (35). Therefore, the Ki-67 "hot spot" represented a selection of a group of malignant cells with a high proliferative activity and potentially associated with a more aggressive biological behavior.

Survival of patients with EMC was calculated according to the Kaplan-Meier method (36). Univariate analysis to identify potential prognostic factors associated with metastasis-free and overall survival was performed with the log-rank test (36) and addressed the following variables: age ($\geq$ 45 or $<$45 years), sex (female or male), tumor size ($\geq$ 10 cm or $<$10 cm), cellularity (high or low), mitotic activity ($>$2 or $\leq$2 per 10 high-power fields), atypical features (presence of anaplasia or rhabdoid phenotype), Ki-67 expression ($\geq$ 10 or

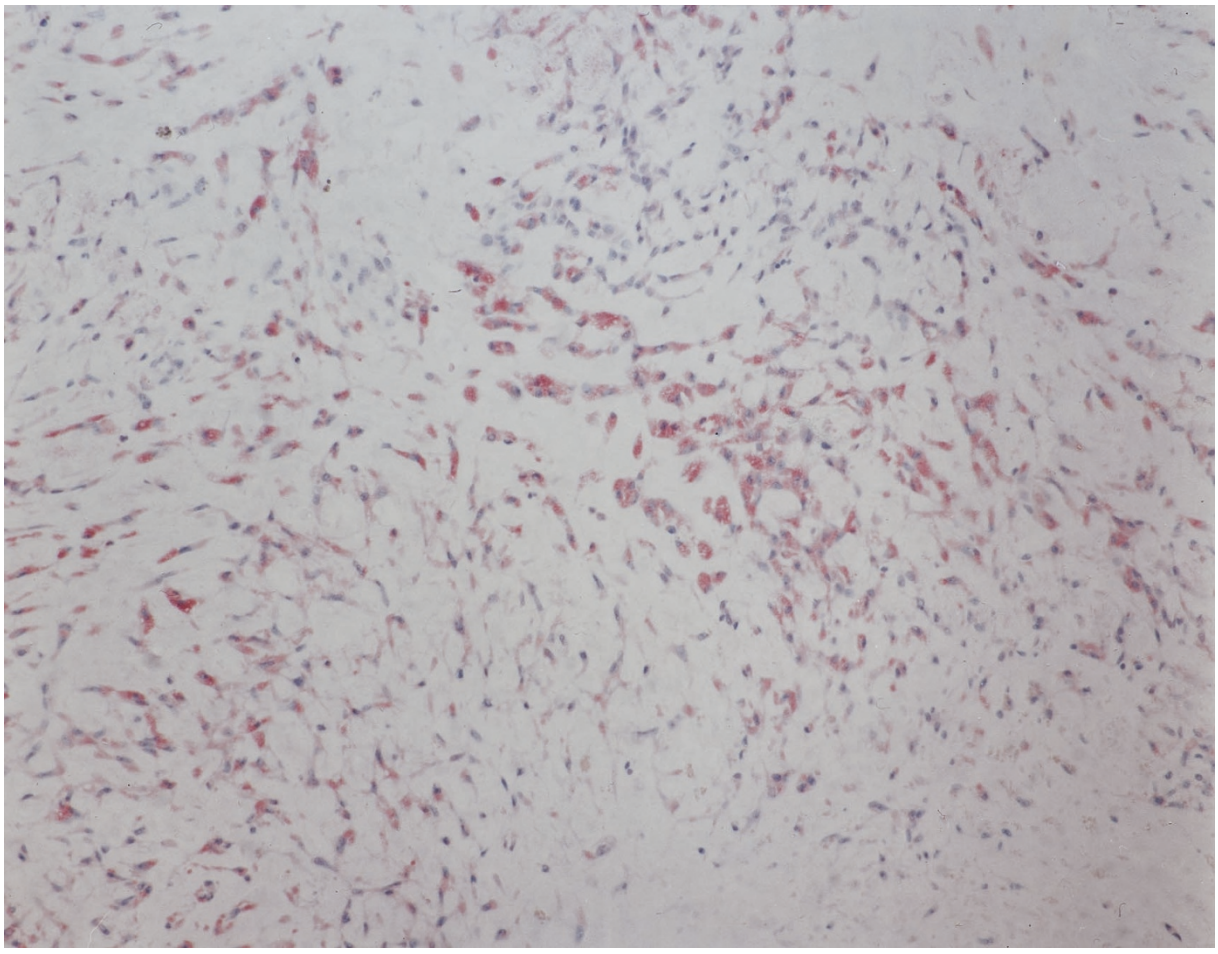

FIGURE 5. Synaptophysin immunoreactivity. This was present in 72% of the tumors.

TABLE 3. Immunohistochemical Findings in 18 Patients With Extraskeletal Myxoid Chondrosarcoma

Marker	Case																	
	1	2	3	4	6	9	10	11	13	14	15	16	17	18	19	20	21	23
Vimentin	+	+	+	+	−	+	+	f+	−	+	+	+	+	+	+	+	+	+
Desmin	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
S-100	−	−	+	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−
EMA	−	f+	−	−	−	+	+	−	−	−	−	+	−	−	+	−	−	−
Cytokeratin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
PCEA	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Synaptophysin	f+	f+	f+	−	−	f+	+	+	−	+	f+	+	−	+	f+	+	−	+
Chromogranin	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
Leu-7	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
α-Smooth muscle actin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Actin muscle-specific	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
MIC2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
GFAP	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−

f, focal.

<10%), Ki-67 "hot spot" expression (≥ 25% or <25%), and DNA ploidy (diploid or nondiploid). Vascular invasion and spontaneous necrosis were not evaluated as potential prognostic factors because of the small number of cases in which these features were found. Type of operation (marginal excision, wide local excision, or amputation) and radiotherapy use were excluded from the analysis because of the lack of standardization in the treatment and the small number of cases in our series. Local recurrence was excluded as an end point for survival analysis because of the few events observed (three cases). All *P* values were two-tailed.

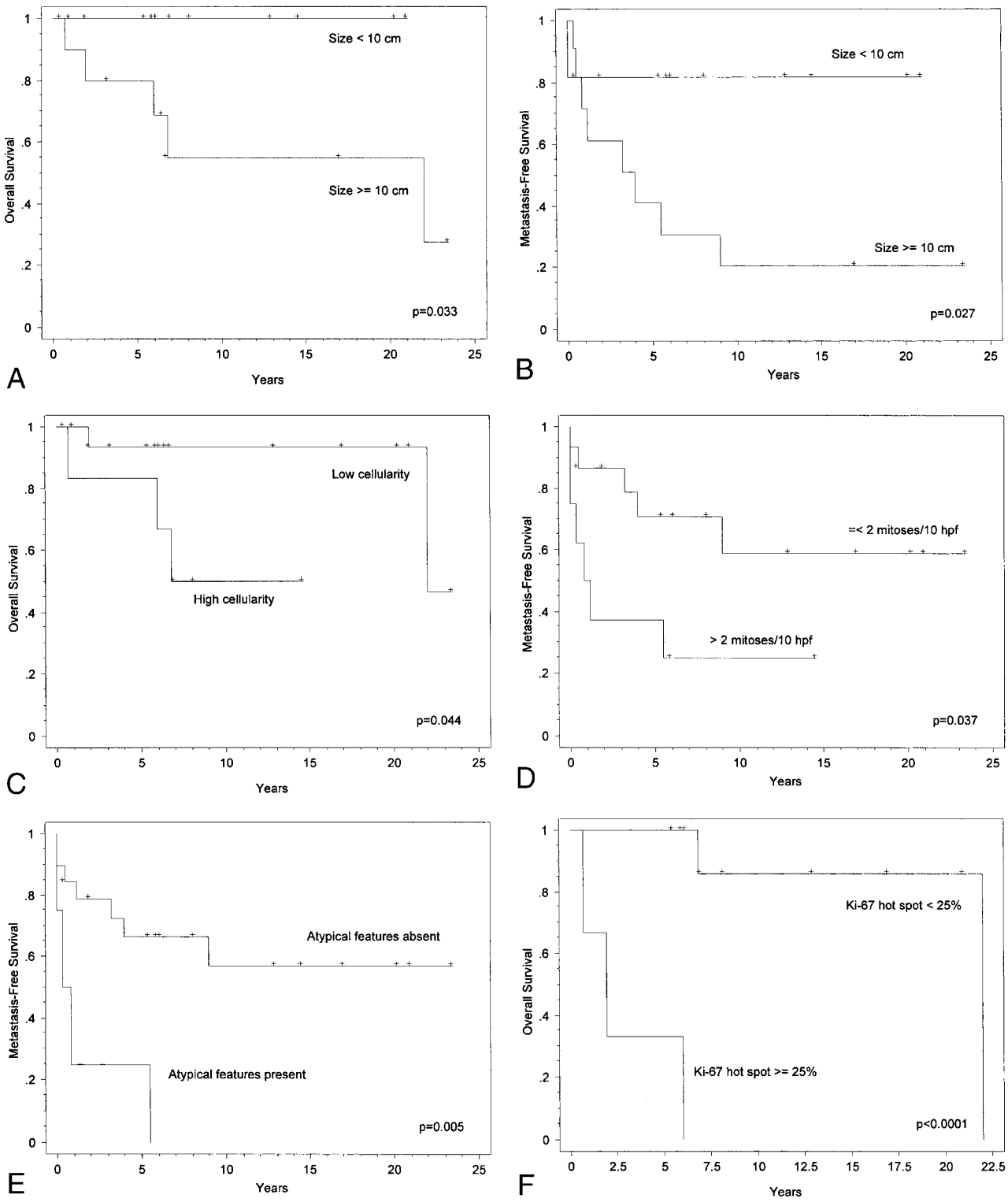

FIGURE 6. Stratified survival curves according to the log-rank test for overall survival and tumor size (**A**), metastasis-free survival and tumor size (**B**), overall survival and cellularity (**C**), metastasis-free survival and mitotic activity (**D**), metastasis-free survival and atypical features (**E**), and overall survival and Ki-67 hot spot activity (**F**).

RESULTS

Clinical Findings

Of the 23 patients, 12 were women and 11 were men; their ages ranged from 22 to 78 years (median, 50). The tumors were located in the lower extremities (19 cases, 83%), upper extremities (two cases), anterior chest wall (one case), and paravertebral area (one case). Among the tumors located in the lower extremities, the thigh was the most common

TABLE 4. Unfavorable Prognostic Factors in Extraskeletal Myxoid Chondrosarcoma by Univariate Analysis

Factor	*P* Value	
	Metastasis-free Survival	Overall Survival
Age (≥45 years old)	0.783	0.126
Male sex	0.077	0.068
Tumor ≥10 cm	0.027	0.033
High cellularity	0.429	0.044
Mitotic activity (>2/10 HPF)	0.037	0.099
Ki-67 ≥10%	0.012	0.563
Ki-67 "hot spot" ≥25%	0.027	<0.0001
Nondiploidy	0.902	0.243
Atypical features*	0.005	0.001

* Defined as the presence of anaplasia or rhabdoid phenotype.
HPF, high-power field.

anatomical location (nine cases), followed by the ankle (four cases), foot (three cases), knee (two cases), and leg (one case). Twenty-two tumors were deep-seated, and one was subcutaneous (case 4). The duration of symptoms before diagnosis ranged from 1 to 60 mo (median, 6 mo), and the presence of a palpable mass was the main complaint in all patients but one, who referred to pain only (case 19).

All patients underwent surgical treatment. Wide local excision was performed in 17 patients (74%), limb amputation in four (17%), and marginal excision in two. All margins were considered negative, two after reexcision. Ten patients also received radiation treatment (median dose, 50 Gy). Of these patients, eight received postoperative radiotherapy, one received preoperative and postoperative radiotherapy, and one received preoperative radiotherapy. One patient received preoperative chemotherapy with MAID (combination of mesna, doxorubicin [Adriamycin], ifosfamide, and dacarbazine; case 15). Follow-up information was obtained on all patients, and the duration of follow-up ranged from 0.5 to 23.5 years (mean, 9.3). Three patients presented with metastatic disease (cases 3, 6, and 15) and eight patients had metastasis between 0.3 and 9 years after the initial diagnosis. Metastases were most common in the lungs, followed by bones, lymph nodes, and soft tissues (Table 2). The primary tumor was discovered in one of these patients (case 3) only 4.3 years after the diagnosis of metastatic disease. Local recurrence developed in three patients (cases 20, 21, and 7) 0.25, 4.8, and 8 years after the initial diagnosis, respectively. Five patients (22%) died of tumor between 0.7 and 22 years after the initial diagnosis; two of these patients had a rapid clinical course, dying in less than 2 years (cases 1 and 9). The remaining 18 patients (78%) were alive at the end of follow-up, six with metastatic disease (Table 2). The 5-, 10-, and 20-year overall survival rates were 91%, 78%, and 78%, respectively. The constancy of cumulative survival between 10 and 20 years was due to the lack of tumor-related deaths during this time interval.

Pathologic Findings

Tumor size ranged from 1.5 to 18.5 cm (median, 9.5). Macroscopically, the neoplasms were usually described as well-circumscribed, tan-gray, multilobulated, gelatinous masses with focal or extensive hemorrhagic areas.

Histologically, all tumors presented a multinodular architecture and were composed of cords or strands of small cells immersed in a myxoid matrix (Fig. 1 and 2). The cells were ovoid or spindle-shaped with a deeply eosinophilic cytoplasm and frequently exhibited hyperchromatic nuclei. Areas composed of large atypical cells with prominent nucleoli were observed in three tumors (cases 1, 3, and 9; Fig. 3). Rhabdoid features were found in two neoplasms (cases 1 and 11). Foci of hyaline cartilage were present in only one neoplasm (case 19); an extensive spindle cell component, fibrosarcoma-like, was present in another (case 4). The average mitotic count was usually low, with 61% of all tumors (14 cases) having less than one mitotic figure per 10 HPF. Eight tumors had more than two mitotic figures per 10 HPF (cases 1, 3, 9, 11, 12, 13, 15, and 18). Spontaneous necrosis was identified in two neoplasms (cases 5 and 9). Necrosis was identified in two other neoplasms, but the patients had received preoperative chemotherapy and radiotherapy (cases 15 and 20, respectively). Vascular invasion was detected in three tumors (cases 1, 4, and 18). The surgical margins were negative in all tumors. In two tumors, the margins were considered positive after excisional biopsy but negative after reexcision. The degree of cellularity was considered low in 16 cases (70%) and high in seven (30%) according to the criteria described above (Table 2; Fig. 4).

Immunohistochemical Findings

Vimentin was expressed in 16 cases (89%). Synaptophysin was expressed in 13 cases (72%; Fig. 5); focal immunoreactivity was present in six cases. Epithelial membrane antigen (EMA) was expressed in a membrane pattern in five cases (28%). S-100 protein nuclear immunostaining was detected in only three cases. Desmin was focally expressed in two cases. Immunoreactivity for chromogranin was present in only one tumor (case 11). Leu-7 showed true reactivity in only one case, but a nonspecific background matrix staining was present in eight cases. Focal glial fibrillary acid protein (GFAP) immunoreactivity was identified in case 15. All tumors lacked immunoreactivity for actin muscle-specific,

smooth muscle actin, cytokeratin, polyclonal carcinoembryonic antigen (pCEA), and MIC2 (Table 3).

Digital Image Analysis

Ploidy results by digital image analysis are listed in Table 2. Nine tumors were diploid, three aneuploid, and one tetraploid. Ki-67 activity ranged from 1 to 45% (mean, 11%), and Ki-67 "hot spot" activity ranged from 3 to 49% (mean, 19%). High Ki-67 and Ki-67 "hot spot" activities were present in cases 1 (45% and 49%, respectively) and 9 (26% and 48%, respectively), and both patients died of disease.

Prognostic Factors

By univariate analysis, tumor size (≥ 10 cm) and Ki-67 "hot spot" activity (≥ 25%) correlated with decreased metastasis-free and overall survival (Fig. 6). High cellularity correlated only with decreased overall survival. High mitotic activity (more than two mitotic figures per 10 HPF) and overall Ki-67 activity ≥10% correlated with decreased metastasis-free survival. Because anaplasia and rhabdoid phenotype were found in a few cases, both features were combined under the term "atypical features" for statistical purposes. The presence of atypical features strongly correlated with metastasis-free and overall survival. A trend to decreased overall survival was observed with high mitotic activity, male sex, and older age, but these features did not reach statistical significance. Ploidy status was not correlated with any adverse outcome (Table 4). The other clinical and histologic factors were not associated with adverse outcomes or could not be analyzed because of the small number of cases studied. Necrosis was excluded as a potential prognostic factor because two of the patients in whom necrosis was found received preoperative radiotherapy or chemotherapy.

DISCUSSION

Our findings indicate that EMC is usually an indolent tumor and most patients survive for long periods, even in the presence of metastatic disease. However, a few patients can have an aggressive clinical course and die shortly after diagnosis. This is exemplified in two of our cases, and they emphasize the importance of recognizing adverse prognostic factors in EMC to optimize treatment and follow-up.

Because of the small number of patients studied, multivariate analysis for the identification of independent prognostic factors could not be performed. Nonetheless, univariate analysis identified some potential markers. Tumor size was an important prognostic factor, as observed in many other neoplasms: all patients in our series who died presented with large tumors (≥ 10 cm at diagnosis) and developed metastatic disease. These findings are in agreement with recent studies by Meis-Kindblom *et al.* (4), who identified large tumor size as an independent prognostic factor by multivariate analysis.

Some reports have shown that high grade or cellularity is correlated with decreased survival (2, 5, 16, 17, 19). However, other reports have disregarded these findings (3, 4). Meis-Kindblom *et al.* (4) studied the impact of cellularity (categorized as low, moderate, or high) as a prognostic factor in EMC and did not find any association between this histologic feature and adverse outcomes. However, no specific criteria for this stratification were provided. Judging by the pictures in that study, we compared our high-cellular cases with the high-cellular cases exemplified by those authors. We identified high cellularity and mitotic activity (more than two mitotic figures per 10 HPF) as potential predictors of an adverse outcome. However, because most of the patients who died of disease or developed metastases had large tumors, the independence of those histopathologic features could not be addressed. Other histologic features that could be associated with adverse outcomes were a rhabdoid phenotype and anaplasia. Rhabdoid cells were present in two neoplasms, and both patients died of disease. Anaplasia was present in three tumors, and two patients died of disease in less than 2 years. These histologic features were combined under the term "atypical features" and strongly correlated with metastasis-free and overall survival. These cases showed striking histologic similarities with those recently described by Lucas *et al.* (37), and the aggressive clinical behavior observed in our cases supports the impression of those authors.

EMC ploidy profile has not been extensively studied (25), and according to our results EMC may show diploid, tetraploid, or aneuploid DNA contents. However, ploidy status did not seem to correlate with any adverse outcome. Ki-67 overall activity (≥ 10%) was associated with metastasis-free survival but not overall survival in our series. Similar findings were observed by Meis-Kindblom *et al.* (4). However, in that study, overall Ki-67 activity (treated as a continuous variable) did not correlate with any adverse event in a multivariate model. High Ki-67 "hot spot" activity (≥ 25%) correlated with both decreased metastasis-free and overall survival. The association between the Ki-67 activity in the "hot spot" area and an adverse prognosis suggests that subclones of cells in EMC with a high proliferative rate might have a high metastatic potential, dictating the overall prognosis (33, 34).

Between 18 and 60% of EMCs express EMA (4, 15, 19, 38). In our series, a distinct membrane staining

was present in only five tumors (28%). In contrast, none of the tumors were immunoreactive for cytokeratin, which has been previously demonstrated by other authors in a few cases of EMC (4, 39–42). These results indicate that the meaning of EMA immunoreactivity needs further elucidation in regard to epithelial differentiation. More importantly, EMA immunoreactivity in EMC may be a problem in the differential diagnosis with other tumors with "chordoid" features, such as chordomas, chondroid chordomas, meningiomas with chordoid features, myoepitheliomas, and chondroid syringomas (15, 42–46). In this regard, clinical information, including tumor location, and negative immunostainings for cytokeratin and actin support the diagnosis of EMC. In addition, a clear membranous epithelial membrane antigen immunoreactivity in EMC occurs in a minority of cases. In meningiomas, epithelial membrane antigen immunoreactivity is usually diffuse and present in almost all cases.

Neuroendocrine differentiation has been reported in tumors with histologic and molecular features of EMC by Chhieng *et al.* (46a). Immunoreactivity for synaptophysin was identified in 72% of the 18 tumors evaluated, and one of these tumors also coexpressed chromogranin. Synaptophysin is a 38-kd transmembrane calcium-binding glycoprotein typically found in neuronal presynaptic vesicles (47, 48) and is expressed by neuroendocrine and neuroectoderm-derived cells (49, 50). Synaptophysin has been shown to be a useful marker of neuroendocrine differentiation when the monoclonal antibody SY38 is used (50). Furthermore, in tissues fixed in formalin, synaptophysin expression can be attenuated, decreasing its sensitivity (51). Chromogranins are a group of acidic proteins present in neurosecretory granules (52) and expressed by most types of neuroendocrine tumors (53). Chromogranin A is widely distributed in neuroendocrine tissues, but its expression depends on the number of neuroendocrine granules present in the cells. Cells with few neuroendocrine granules may show false-negative immunostaining, and this has been demonstrated by variable expression of this marker in small cell carcinomas of the lung, neuroblastomas, Merkel cell carcinomas, and a few other tumors (54). Our results not only support the observations of Chhieng *et al.* but also show that neuroendocrine differentiation is common in EMC.

Our study indicated that EMC is frequently indolent and is associated with long-term survival, even with metastatic disease. However, an aggressive clinical course occurs in a few cases. Large tumor size, high cellularity, anaplasia or rhabdoid features, high Ki-67 expression, and high mitotic activity seem to be associated with adverse outcomes, but the independence of these clinicopathologic features in relationship to the prognosis could not be addressed. In this regard, we suggest a closer follow-up for patients with any of these features.

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