Prognostic Significance of Macrophage Infiltration in Leiomyosarcomas

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Abstract

Purpose: Macrophages are migratory cells that are frequently recruited to the site of tumors. Their presence is associated with poor clinical outcome in a variety of epithelial malignancies. The aim of this study is to examine the prognostic significance of tumor-associated macrophages in sarcomas.

Experimental Design: Global gene expression profiling data of a series of soft tissue tumors were analyzed for macrophage-associated gene expression. Immunohistochemistry on tissue microarrays containing leiomyosarcoma cases with known clinical outcome was used to verify the presence of macrophages and to examine the relationship between tumor-associated macrophages and clinical outcome.

Results: Gene expression profiling revealed high-level expression of several macrophage-associated genes such as *CD163* and *CD68* in a subset of leiomyosarcomas, indicating the presence of variable numbers of tumor-infiltrating macrophages. This was confirmed by CD68 and CD163 immunostaining of a tissue microarray containing 149 primary leiomyosarcomas. Kaplan-Meier survival analysis showed that high density of tumor-infiltrating macrophages as identified by CD163 or CD68 staining is associated with a significantly worse disease-specific survival in nongynecologic leiomyosarcomas, whereas leiomyosarcomas arising from the gynecologic tract showed no significant association between macrophage infiltration and survival. The presence of tumor necrosis did not correlate significantly with outcome.

Conclusions: An increased density of CD163- or CD68-positive tumor-infiltrating macrophages is associated with poor outcome in nongynecologic leiomyosarcomas. This may help the clinical management of patients with leiomyosarcomas.

Macrophages are monocyte-derived migratory cells that participate in a variety of physiologic and pathologic processes. They are best characterized for their roles in inflammation, immune response, and tissue remodeling. There is an increasing body of evidence to suggest that macrophages also play an important role in tumor development and progression, particularly in the cases of epithelial malignancies (1–5). Studies on various types of carcinomas have shown that macrophages are often recruited to the site of tumors. These tumor-associated macrophages can interact with the neoplastic cells through the release of various growth factors and cytokines, which can contribute to cancer initiation and progression by providing a survival advantage for the tumor cells and enhancing angiogenesis (2, 3, 6). Furthermore, macrophages also release a number of proteases that assist in the breakdown of various connective tissue layers, thereby allowing cancer cells to enter into different tissue compartments. In keeping with the experimental evidence for the importance of macrophages in tumor progression, the presence of a high number of tumor-associated macrophages in various types of carcinomas, including breast, prostate, endometrial, kidney, and bladder carcinomas, as well as in lymphomas, is associated with poor prognosis (7–12).

Sarcomas are a heterogeneous group of malignant tumors that are derived from cells of mesenchymal origin. They can arise in diverse body sites and can afflict patients from a wide age range. There is often a nonneoplastic mesenchymal component that includes fibroblasts, blood vessels, and inflammatory infiltrates, including macrophages. Little is currently

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known regarding the significance of tumor-associated macrophages in sarcomas.

For most types of sarcomas, there are currently no well-established prognostic factors beyond grade, size, and site that can assist in the management of patients, especially in terms of follow-up and the necessities for multimodality treatments (13). The identification of novel prognostic factors, particularly ones that are relevant to the oncobiology of sarcomas, will therefore be of clinical value in the management of sarcoma patients.

In the present study, using gene expression profiling, we observed high-level expression of a number of macrophage-associated genes in a subset of leiomyosarcomas and subsequently examined the prognostic significance of tumor-infiltrating macrophages as identified by CD68 or CD163 immunostains on tissue microarrays. We found that the density of intratumoral CD68- or CD163-positive macrophages in primary leiomyosarcomas arising outside of the gynecologic tract locations is inversely correlated with patient outcome.

Materials and Methods

Tumor samples. Fresh frozen samples of 51 soft tissue tumors were obtained from surgical specimens resected at nine centers across the United States, Canada, and the Netherlands. These included 10 tenosynovial giant cell tumors, 8 gastrointestinal stromal tumors, 8 cases of desmoid-type fibromatosis, 8 leiomyosarcomas, 7 synovial sarcomas, 5 solitary fibrous tumors, and 5 dermatofibrosarcoma protuberans. All cases with exception of the eight leiomyosarcomas have been previously analyzed on cDNA microarrays (14–18) but are reanalyzed for this study on new oligonucleotide-based Human Exonic Evidence Based Oligonucleotide (HEEBO) arrays. The clinicopathologic features of the eight leiomyosarcomas are shown in Supplementary Table S1. Paraffin-embedded samples of 149 specimens from 149 different patients with leiomyosarcomas were collected from 107 hospitals and laboratories across the United States, Canada, and the Netherlands (Table 1). The 149 specimens represent biopsy or resection of the primary disease, and the date of the diagnosis ranged from 1985 to 2005. Clinical characteristics and disease-specific survival (minimum follow-up of 6 months) were available for all 149 cases. None of these patients received neoadjuvant treatment (chemotherapy and/or radiotherapy). All of the cases were centrally reviewed (C.H.L. and I.E.), and the diagnosis of leiomyosarcomas was confirmed based on histologic and immunohistochemical evaluations. Leiomyosarcomas were defined as tumors growing with a fascicular architecture showing obvious histologic features of smooth muscle differentiation (spindle cell neoplasms with well-defined eosinophilic cytoplasm containing longitudinal striations and blunt-ended nuclei). Tumors with less obvious histologic features of smooth muscle differentiation required, at a minimum, either both focal smooth muscle actin and desmin staining, or strong diffuse smooth muscle actin staining to be included in the study. All cases were negative for KIT and myogenin, thus excluding the possibility of contaminating gastrointestinal stromal tumors and rhabdomyosarcomas cases in the study. The Fédération Nationale des Centres de Lutte Contre le Cancer (FNCLCC) grading system, which focuses on the three variables—mitotic index, necrosis, and cell differentiation—was used to grade all tumors arising outside of the gynecologic tract (19). Tumors arising in the gynecologic tract were graded based on the degree of cellular differentiation: (*a*) well differentiated, (*b*) moderately differentiated, and (*c*) poorly differentiated. The institutional review board of Stanford University approved this study.

HEEBO gene arrays. The HEEBO microarray used in the study contained 44,544 70-mer probes that were designed using a transcriptome-based annotation of exonic structure for genomic loci.[5] After confirmation of histology and the presence of viable tumor by frozen section, specimens were homogenized in Trizol reagent (Invitrogen), and total RNA was extracted. The total RNA was reverse transcribed into cDNA using a mixture of oligo dT (Operon; high-performance liquid chromatography purified) and random hexamer (Amersham) primers with incorporation of amino allyl-dUTP (Ambion). Cy3 and Cy5 dyes (Amersham) were used for indirect labeling of the cDNA from reference RNA (Stratagen; Universal human reference RNA) and cDNA from tumor specimens, respectively. Microarray hybridization and washing was done using standard procedures (20, 21). Microarrays were scanned on a GenePix 4000 microarray scanner, and fluorescence ratios (tumor/reference) were calculated using GenePix software. Only spots with a ratio of signal over a background of at least 1.3 in the Cy5 and 1.5 in the Cy3 channel were included. Gene centering was applied to the

Table 1. Summary of 149 primary LMS used in tissue microarray construction

	Gynecologic LMS	Nongynecologic LMS
Total number of cases	76	73
Age at time of diagnosis (y)		
Average	50.7	53.5
≤40	7	11
41-50	29	20
51-60	33	14
>60	7	28
Sex		
Male	NA	31
Female	76	42
Tumor location		
Gynecologic tract (n = 76)		
Uterus	70	NA
Vagina	3	NA
Others	3	NA
Nongynecologic tract (n = 73)		
Retroperitoneum/ abdomen/pelvis	NA	25
Soft tissue limbs	NA	23
Genitourinary system	NA	7
Gastrointestinal system	NA	6
Bone	NA	4
Inferior vena cava	NA	2
Others	NA	6
Tumor size (cm)*		
Average	10.1	9.9
<2	0	1
2-5	11	23
5.1-10	33	21
10.1-20	23	21
>20	2	4
Histologic grade †		
1	20	19
2	38	35
3	18	12

Abbreviations: LMS, leiomyosarcoma; NA, not applicable.
*Tumor size was not available in seven cases of gynecologic leiomyosarcomas and three cases of nongynecologic leiomyosarcomas.
†Tumor grade was assigned based on cellular differentiation for gynecologic leiomyosarcomas and the FNCLCC grading system for nongynecologic leiomyosarcomas; grade was not available in seven cases of nongynecologic leiomyosarcomas.

[5] http://www.microarray.org/sfgf/

expression values for this series of tumors. Our current analysis was restricted to a list of genes (Supplementary Table S2) that were previously implicated in tumor cell–macrophage interaction or are expressed immunohistochemically by macrophages (1–5), and only genes with >80% available good data were analyzed further by hierarchical clustering (22). The complete gene array data set is available through the accompanying website (23).[6]

Tissue microarrays. Tissue microarrays (TA-121 and TA-201) were constructed as described previously using a manual tissue arrayer (Beecher Instruments; ref. 24). For each specimen, two pathologists reviewed the H&E slides. For each leiomyosarcomas, a block with viable tumor was chosen, similar to the selection of tumor regions for mitotic counts. Scarred, myxoid, and hypocellular areas were avoided. A 0.6-mm tissue core was taken from these areas in each of the blocks and inserted into the TA-201 tissue microarray block. For the 23 of the 149 cases of leiomyosarcomas included in TA-121, duplicate 0.6-mm tissue cores were taken. The final tissue microarrays contained one or more representative cores from all 149 tumors along with samples of normal smooth muscle from the gastrointestinal tract, uterus, and bladder.

Immunohistochemistry. Antibodies used were as follows: smooth muscle actin (mouse monoclonal, 1:200, Sigma antibody; no antigen retrieval; Dako Autostainer), desmin [mouse monoclonal, 1:80, Dako antibody; Ventana Mild Antigen Retrieval (pH 8.0); Ventana Autostainer], KIT [rabbit polyclonal, 1:50, Dako antibody; Dako pressure cooker antigen retrieval (pH 6.0); Ventana Autostainer], myogenin [mouse monoclonal, 1:200, Dako antibody; Dako pressure cooker antigen retrieval (pH 6.0); Dako Autostainer], CD68 [mouse monoclonal, 1:1,600, Dako Antibody; Ventana Mild Antigen Retrieval (pH 8.0); Ventana Autostainer], and CD163 [mouse monoclonal, 1:100, Novocastra Antibody; Ventana Mild Antigen Retrieval (pH 8.0); Ventana Autostainer]. For the macrophage markers (CD68 and CD163), only staining in tumor-infiltrating macrophages was considered, and staining of the tumor cells (which was typically much weaker if present) was not included in our current analysis. A tumor-infiltrating macrophage identified by CD68 or CD163 was defined as a cell with oval to round nuclei that possess fine dendritic processes, showing strong membranous/cytoplasmic staining but no nuclear staining. The quantification for CD68 and CD163 immunostaining was done visually as follows: score of 1 (sparse infiltrates) for ≤5 positively stained macrophages per 0.6-mm tumor core, score of 2 (moderate infiltrates) for >5 but ≤25 positively stained macrophages per 0.6-mm tumor core, and score of 3 (dense infiltrates) for >25 positively stained macrophages per 0.6-mm tumor core. Cores in which no diagnostic material was present or with equivocal staining results were omitted from further analysis.

Data analysis. Scoring results were combined using Deconvoluter 6 and TMA-Combiner 7 programs (25). The digital images, collected using a computerized microscope (BLISS; Bacuslabs), are available for all stained cores on all 149 patients through the accompanying Web site.[7] Kaplan-Meier analysis was used to show survival curves with log-rank test to compare survival between two (or more) different groups. Student's t test was used for comparison of the demographics data wherever appropriate. A P value of <0.05 was considered significant.

Results

Gene expression profiling of soft tissue tumors. Global gene expression profiling using oligonucleotide microarrays (HEEBO; Stanford) was done on 51 cases of soft tissue tumors, including 10 tenosynovial giant cell tumors, 8 gastrointestinal stromal tumors, 8 cases of desmoid-type fibromatosis, 8 leiomyosarcomas, 7 synovial sarcomas, 5 solitary fibrous tumors, and 5 dermatofibrosarcoma protuberans. The relative expression levels of selected macrophage-associated genes and genes known or suspected to be of importance in tumor-macrophage interaction are shown in Fig. 1 and revealed variably high levels of expression of many of these genes in leiomyosarcomas (Fig. 1; refs. 1–5). The only soft tissue tumor with a stronger signature for these genes was tenosynovial giant cell tumors, a tumor type primarily comprised of large numbers of macrophages recruited by small numbers of neoplastic cells (18). This identified leiomyosarcomas as a type of sarcoma that may possess a high amount of tumor-associated macrophages at least in a subset of cases.

Clinicopathologic features of the leiomyosarcoma cases. A series of 149 paraffin-embedded primary leiomyosarcoma tissue samples collected from various centers across North America was used for the construction of the tissue microarray. The clinicopathologic features of the tumors are summarized in Table 1. Fifty-one percent of the primary leiomyosarcomas in the current series occurred along the gynecologic tract with the great majority arising from the uterus (gynecologic leiomyosarcomas). The remaining 49% of the leiomyosarcomas occurred in superficial and deep soft tissues, bone, and other visceral structures (nongynecologic leiomyosarcomas). The average age of the patients at the time of initial diagnosis was 52 years (53.5 years for nongynecologic leiomyosarcomas and 50.7 years for gynecologic leiomyosarcomas). Among the nongynecologic leiomyosarcomas, there was a slight predominance of female patients in our series, with a female to male ratio of ~1.4:1. The average size of the primary tumor was 10 cm, and the tumor sizes were similar between gynecologic and nongynecologic groups. Seventy-one percent of the leiomyosarcomas are pathologically high-grade tumors, and tumor necrosis was histologically identified in 38% of the cases. Disease-specific survival data with a median follow-up of 3.1 years were available for all 149 patients. Except for 7 patients who succumbed to the disease within 6 months of the initial diagnosis, all remaining cases had a minimum follow-up period of >6 months. The clinical stages of the tumors were not available for our current series.

CD68 and CD163 immunostaining in leiomyosarcomas. To identify and quantify the amount of tumor-associated macrophages in leiomyosarcomas, we used antibodies against two commonly expressed antigens on macrophages—CD68 and CD163. CD68 and especially CD163 are relatively specific markers for macrophages and monocytes (26–28). Although CD68 and CD163 can both be expressed in macrophages and their precursor monocytes, not all monocytes express both markers concomitantly (29). Representative images of leiomyosarcomas with CD68- or CD163-positive tumor-infiltrating macrophages are shown in Fig. 2. The staining signal was located in the membrane and cytoplasm but not in the nucleus. As shown in Fig. 2, some leiomyosarcomas were extensively infiltrated by macrophages in a dispersed manner, whereas others showed only sparse macrophage infiltrates. The spatially dispersed nature of macrophage infiltration makes their detection on routine H&E sections difficult. Nearly all leiomyosarcomas contained some amount of either CD68- or CD163-positive macrophages in their tumor stroma. The results of the immunostaining are summarized in Table 2. Among the 23 cases of leiomyosarcomas included on TA-121 with

[6] http://genome-www5.stanford.edu

[7] http://tma.stanford.edu/tma_portal/LMS_macrophages

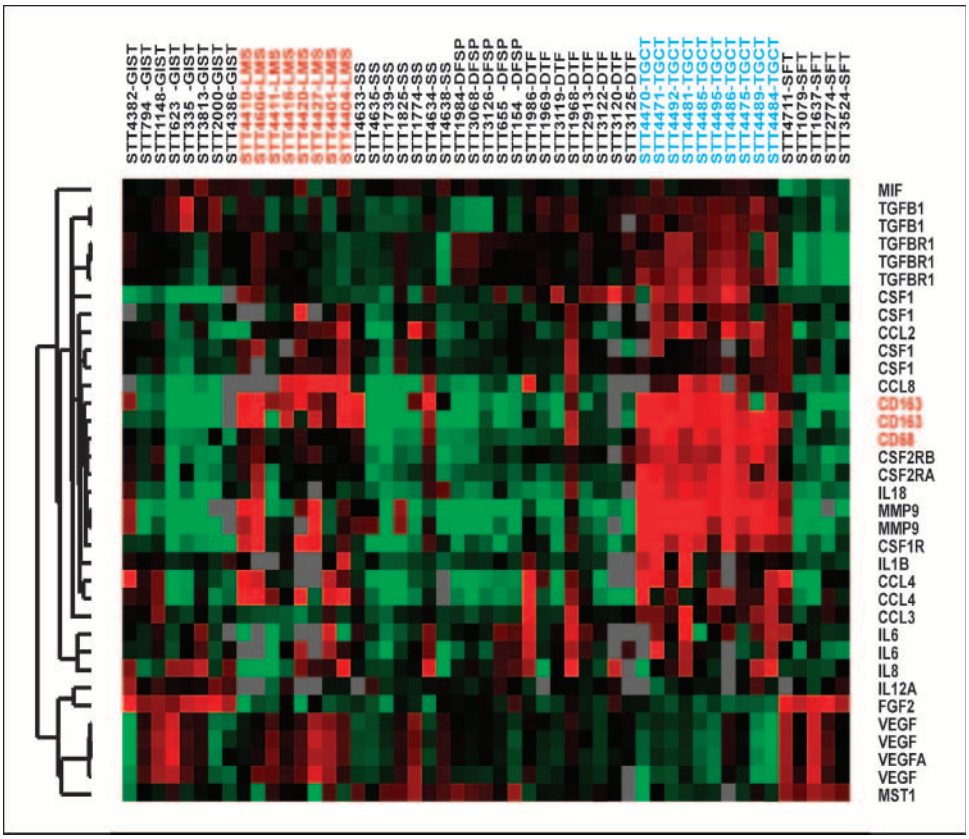

Fig. 1. The expression profiles and hierarchical clustering of selected macrophage markers and genes implicated in macrophage – tumor cell interaction. LMS, leiomyosarcomas; GIST, gastrointestinal stromal tumors; DFSP, dermatofibrosarcoma protuberans; DTF, desmoid-type fibromatosis; SFT, solitary fibrous tumors; SS, synovial sarcomas; TGCT, tenosynovial giant cell tumors.

duplicate cores, all CD163 macrophage density scores were concordant between the duplicate cores except for one case in which one of the two cores was nonscorable. In addition, 20 of the 22 scorable cases showed concordant CD68 density scores of the duplicate cores. The two cases with discordant results both ranged from sparse to moderate density of CD68-positive macrophage infiltrates in the duplicate cores. The higher score from the duplicate cores was used for further analysis. To further determine whether tissue microarray cores are representative of the entire lesion, CD163 immunostaining was done on whole tissue sections of selected cases of leiomyosarcomas that showed dense or sparse CD163-positive macrophage infiltrates. There seems to be a high degree of spatial uniformity in the intratumoral macrophage density (Supplementary Fig. S1), in keeping with the highly concordant scoring results observed between the duplicate cores.

Forty-four percent of the leiomyosarcomas in our series showed dense infiltration (score of 3) by CD163-positive macrophages. In contrast, 26% of the leiomyosarcomas in our series contained a dense CD68-positive macrophage infiltrate. Of note, CD68 and CD163 staining yielded discrepant scoring results in some cases, and a summary of their comparison is shown in Supplementary Table S3. Thirty-one of the 65 cases of leiomyosarcomas that were found to have dense CD163-positive macrophage infiltrates were found to have sparse (n = 7) or moderate (n = 24) CD68-positive macrophage infiltrates. Six of the 39 cases showing dense CD68-positive macrophage infiltrates were found to have moderate CD163-positive macrophage infiltrates with all other cases also showing dense CD163-positive macrophage infiltrates. The distribution of the staining results was similar between gynecologic and nongynecologic leiomyosarcomas for CD163 (Table 2). In contrast, nongynecologic leiomyosarcomas contained more tumors showing dense CD68-positive macrophage infiltrates than gynecologic leiomyosarcomas. None of the leiomyosarcoma tumor cells showed strong staining for CD68 or CD163 in our series.

Tumor-associated macrophages and patient disease-specific survival. Kaplan-Meier survival analysis was done to determine the prognostic significance of tumor-infiltrating macrophages in gynecologic and nongynecologic leiomyosarcomas. Although global gene expression analysis and comparative genomic hybridization studies that compared gynecologic and nongynecologic leiomyosarcomas have revealed relatively little difference between them (30 – 32), these two groups of leiomyosarcomas are generally regarded as different disease entities as they are diagnosed using different histologic criteria, staged using different systems, and treated differently. The densities of tumor-infiltrating macrophages as identified by antibodies against CD68 and CD163 in leiomyosarcomas were graded semiquantitatively into three levels using the criteria described in Materials and Methods. For gynecologic leiomyosarcomas, Kaplan-Meier analysis showed no significant association between the densities of macrophage infiltrates and disease-specific survival based on CD68 immunostaining (P = 0.53) or CD163 immunostaining (P = 0.35; Fig. 3A-B). In contrast, Kaplan-Meier survival analysis based on the densities of CD68- or CD163-positive tumor-associated macrophages in nongynecologic leiomyosarcomas all showed statistically significant association with disease-specific survival (Fig. 3C-D). The semiquantitative grading of CD163-positive macrophages seemed to be better at separating the different grades of tumors

into more distinct survival subgroups than the semiquantitative grading of CD68-positive macrophages. Patients with nongynecologic leiomyosarcomas showing dense, moderate, and sparse CD163-positive macrophages had an estimated 5-year disease-specific survival of ~40%, 70%, and 100%, respectively (P = 0.002). The histologic presence of tumor necrosis in both nongynecologic and gynecologic leiomyosarcomas showed no significant prognostic association with disease-specific survival (Supplementary Fig. S2). Kaplan-Meier survival analysis of the nongynecologic leiomyosarcomas based on the FNCLCC grading system showed a trend toward poorer outcome with higher grade, which did not reach statistical significance (P = 0.17; Supplementary Fig. S3).

These results showed that higher density of macrophage infiltration as shown by CD68 immunostaining and, in particular, by CD163 immunostaining is a negative predictor of patient survival in nongynecologic leiomyosarcomas, and this significant association with survival is not related to the presence of tumor necrosis. In contrast, although some gynecologic leiomyosarcomas can contain dense intratumoral CD68- or CD163-positive macrophage infiltrates, the degree of infiltration does not seem be related to a difference in disease-specific survival for the patients.

Discussion

Using gene expression profiling, we observed in leiomyosarcomas variable levels of expression for several macrophage-associated genes known or suspected to be important in tumor-macrophage interactions. The mRNA expression levels for macrophage marker genes, such as *CD163*, found in some leiomyosarcomas were similar to that found in tenosynovial giant cell tumor, which is known to contain an abundance of infiltrating monocytes/macrophages within their tumor stroma (18). This suggested that a subset of leiomyosarcomas likely contains a high number of tumor-associated macrophages, a finding that can be easily overlooked on conventional H&E stains. Subsequent immunohistochemical staining confirmed that a significant subset of leiomyosarcomas contains dense CD68- or CD163-positive macrophage infiltrates. More importantly, patients with nongynecologic leiomyosarcomas showing a higher density of intratumoral infiltration by CD68- or CD163-positive macrophages were found to have shorter 5-year disease-specific survival when compared with patients with tumors showing less dense CD68- or CD163-positive macrophages. In contrast, leiomyosarcomas arising from the gynecologic tract did not show a significant correlation between the

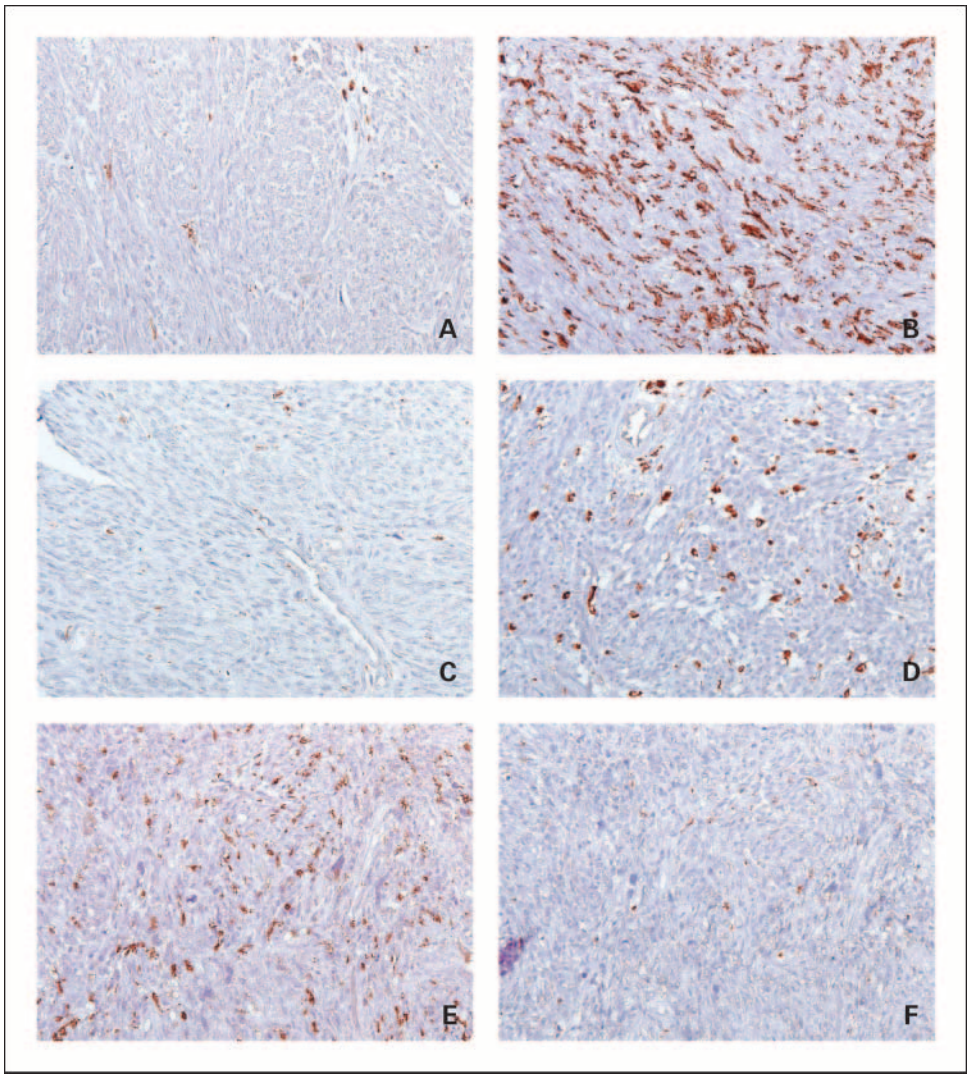

Fig. 2. Representative images showing leiomyosarcomas with sparse infiltrate of CD163-positive macrophages (*A*; case no.79), dense infiltrate of CD163-positive macrophages (*B*; case no.224), sparse infiltrate of CD68-positive macrophages (*C*; case no.1), dense infiltrate of CD68-positive macrophages (*D*; case no.62), dense infiltrate of CD163-positive macrophages (*E*; case no.161), and sparse infiltrate of CD68-positive macrophages (*F*; case no.161).

Table 2. Summary of CD163 and CD68 immunohistochemistry results in 149 primary leiomyosarcomas

	Gynecologic LMS	Nongynecologic LMS	All LMS
CD68 score			
1	26 (34%)	22 (31%)	48 (33%)
2	36 (47%)	24 (34%)	60 (41%)
3	14 (19%)	25 (35%)	39 (26%)
CD163 score			
1	10 (14%)	8 (10%)	18 (12%)
2	32 (44%)	34 (45%)	66 (44%)
3	31 (42%)	34 (45%)	65 (44%)

NOTE: Score 1, sparse infiltrates; score 2, moderate infiltrates; score 3, dense infiltrates.

density of macrophage infiltration and disease-specific survival. This apparent association between disease-specific survival and the amount of CD68- or CD163-positive tumor-associated macrophages in nongynecologic leiomyosarcomas is independent of tumor necrosis as the presence of tumor necrosis showed no significant correlation with patient outcome.

Leiomyosarcoma is a malignant mesenchymal tumor that exhibits evidence of smooth muscle differentiation and accounts for ~10% of all sarcomas. It is an aggressive disease with a propensity for systemic spread and is associated with high recurrence rates and a poor prognosis overall (33–35). The management of leiomyosarcoma is further complicated by the lack of good prognosticators for the disease. Leiomyosarcoma most frequently occurs in the gynecologic tract, particularly the uterus, and in soft tissues. Although gynecologic and nongynecologic leiomyosarcomas both display the same line of cellular differentiation and similar gene expression signatures (30, 32, 36), there are some differences between them. In the gynecologic tract, benign smooth muscle tumors are orders of magnitude more common than leiomyosarcomas, whereas at deep soft tissue sites, benign smooth muscle tumors are rare. Histologically, a higher level of mitotic activity is required for a diagnosis of leiomyosarcomas in the gynecologic tract, compared with soft tissue leiomyosarcomas. Gynecologic leiomyosarcomas also tend to express hormone receptors, such as estrogen receptor and progesterone receptor, more frequently than extrauterine leiomyosarcomas (37). In addition, the clinical staging systems commonly used for these two groups of leiomyosarcomas also differ. The findings presented here suggest a further potential difference between gynecologic and

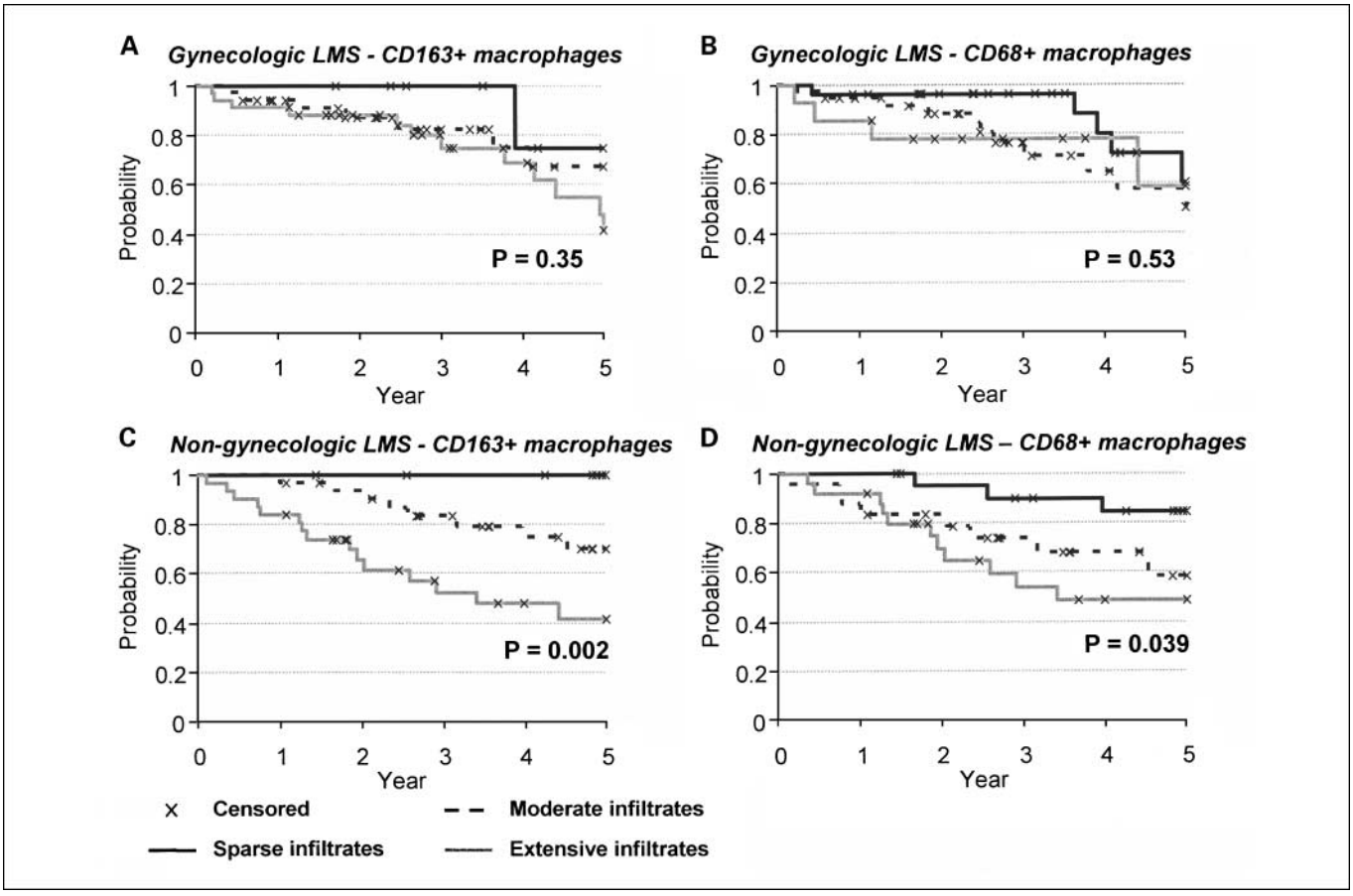

Fig. 3. *A,* Kaplan-Meier survival curves of gynecologic leiomyosarcomas containing varying density of CD163-positive macrophage infiltrates. *B,* Kaplan-Meier survival curves of gynecologic leiomyosarcomas containing varying density of CD68-positive macrophage infiltrates. *C,* Kaplan-Meier survival curves of nongynecologic leiomyosarcomas containing varying density of CD163-positive macrophage infiltrates. *D,* Kaplan-Meier survival curves of nongynecologic leiomyosarcomas containing varying density of CD68-positive macrophage infiltrates.

nongynecologic leiomyosarcomas. Although both gynecologic and nongynecologic leiomyosarcomas can possess similar levels of intratumoral macrophage infiltrates in comparable proportions of the cases, a higher density of macrophage infiltrates is associated with a significantly worse disease-specific survival only for nongynecologic leiomyosarcomas but not for gynecologic leiomyosarcomas.

The prognostic significance of tumor-associated macrophages have been shown in several other tumor types, including carcinomas, melanomas, and lymphoma (7, 8, 10–12, 38–40), but the immunohistochemical identification of tumor-associated macrophages in these other studies involved the use of antibodies against CD68. In our current study, we also used an antibody against CD163 to identify these tumor-associated macrophages. Interestingly, our semiquantitative three-tier grading of the density of CD163-positive tumor-infiltrating macrophages allowed for better prognostic distinction than grading of CD68-positive tumor-infiltrating macrophages. The difference in staining patterns may be due to the phenomenon of monocyte/macrophage heterogeneity (29), as different subgroups of tumor-associated macrophages were identified by antibodies against CD68 and CD163. However, the precise pattern of expression of CD163 and CD68 across the different subtypes of monocytes/macrophages has not been reported in the literature. Alternatively, it is also possible that the tumor-associated macrophages present in leiomyosarcomas simply express higher levels of CD163 relative to CD68, and the antibody used against CD163 was therefore able to outline the macrophages more clearly, thereby allowing for a more precise assessment of the density of tumor-associated macrophages. Our findings also suggest that the intratumoral spatial distribution of macrophage infiltrates in the viable and cellular regions of the tumor is relatively uniform, as highly concordant density scores was found in a subset of cases represented by duplicate cores in this current series, and CD163 whole tissue section immunostaining done on selected cases of leiomyosarcomas showed a spatially uniform pattern of macrophage infiltration.

For the nongynecologic leiomyosarcomas included in our current series, the FNCLCC grading system was not a statistically significant predictor for disease-specific survival, although there was a trend for high-grade tumors to be associated with lower 5-year disease-specific survival when compared with the low-grade tumors ($P = 0.17$). This perhaps reflects the fact that the FNCLCC grading system was not specifically designed for the purpose of prognostication in leiomyosarcomas. In contrast, our semiquantitative assessment of the density of tumor-associated CD68- or CD163-positive macrophages showed prognostic significance. Because of the lack of information on the clinical stage of the disease in our current series, we were not able to further assess the relationship between the amount of macrophage infiltrates and the presence of disseminated disease.

Although not directly examined in this study, our findings here imply the presence of functional interaction between macrophages and tumor cells of nongynecologic leiomyosarcomas. In contrast to gynecologic leiomyosarcomas, it is plausible that the neoplastic cells of a subset of nongynecologic leiomyosarcomas have acquired the ability to recruit and regulate the activities of macrophages to facilitate its tumor progression. Along this line, several studies have shown the importance of such functional interplays between tumor-associated macrophages and breast cancer cells. This includes a number of paracrine interactions between macrophage and the cancer cells that can further promote tumor angiogenesis, invasion, and metastasis (39, 41–43).

We describe here a significant association between the density of CD68- and CD163-positive tumor-associated macrophages in nongynecologic leiomyosarcomas and patient survival. The realization that a semiquantitative assessment of the density of tumor-infiltrating macrophages can be used to predict the behavior of nongynecologic leiomyosarcomas is clinically important, particularly given our current lack of good prognostic markers for this disease and the progressive clinical course that a significant number of leiomyosarcomas pursue. This association also suggests that there may be important functional interaction between the macrophages and leiomyosarcoma tumor cells. Ultimately, it is possible that therapy aimed at disrupting key functional interactions between tumor cells and macrophages may be used to attenuate the malignant behavior of the tumor and prolong survival in a subset of leiomyosarcomas.

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