

Prognostic Significance of Ezrin Expression in Liposarcoma

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Background : Ezrin has been reported to be involved in the metastasis of solid tumors in both an animal model and clinical trials. However, questions remains as to whether an ezrin expression is an independent predictor of the event-free survival of liposarcoma patients.

Methods : We analyzed 85 liposarcoma patients without metastasis at the time of presentation. We performed immunohistochemistry with anti-ezrin antibody, and we analyzed the association of an ezrin expression with the clinicopathological variables and event-free survival. **Results :** Twenty-nine patients (34.1%) showed an ezrin expression. Among the 30 low-grade liposarcoma patients, only one patient showed ezrin positivity. The patients who had an ezrin expression were found to be at a significantly increased risk for metastasis compared with the patients who had no ezrin expression (risk ratio: 3.4, 95% confidence interval: 1.3-9.1). The 10-year metastasis-free survival rate was 26.9% for the patients with an ezrin expression and 86.7% for the patients without an ezrin expression. The ezrin expression rate increased with an advanced tumor grade and stage. **Conclusions :** An ezrin expression is an independent predictor of distant metastasis for liposarcoma. Thus, ezrin has the potential to provide additional prognostic information and to be a novel target for the development of new adjuvant therapies for treating the patients who suffer from liposarcoma.

Key Words : Liposarcoma; Metastasis; Ezrin

Liposarcoma is one of the most common soft tissue sarcomas, and it accounts for about 10-20% of all the sarcomas of adults.¹ These tumors are characterized by wide spectrum of morphological features and a diverse anatomical distribution.² Liposarcomas can be divided into 3 main subtypes: well-differentiated liposarcoma, myxoid/round cell liposarcoma and pleomorphic liposarcoma. Each subtype corresponds to the tumor grade, except for myxoid/round cell liposarcoma.

The most important prognostic factor for liposarcoma is the stage of the tumor,³ and the tumor grade, size and depth and the presence of metastasis are determinants of the stage.⁴ Other prognostic variables, such as mitosis, necrosis and a round cell component, are the factors that determine the grade of a tumor.⁵ Stage I and stage IV liposarcoma are well known to have predictable clinical behavior. However, even when all the known prognostic markers are taken into account, the prognosis of stage II and III liposarcoma patients is still unpredictable.

The variable clinical behavior of liposarcoma suggests the possibility that other factors mediate local and systemic disease progression. Recent molecular biological studies have identified

ezrin as a key component of metastasis, and alteration of its expression has been shown to mediate many changes in the metastasis-associated cell surface signals.⁶ There is evidence from retrospective human studies suggesting that the ezrin expression is correlated with progression of such tumors as osteosarcoma, rhabdomyosarcoma, melanoma and ovarian and pancreas cancer.⁷ However, any studies about liposarcoma are rare.⁸

To evaluate the possibility of using the ezrin expression as a new prognostic marker, we determined the ezrin expression in specimens of primary liposarcoma by performing immunohistochemical staining, and we correlated its expression with the patients' outcome.

MATERIALS AND METHODS

Patients and tumor material

All the consecutive liposarcomas that were diagnosed at our institute from 1988 to 2002 were reviewed in regard to the clin-

icopathological data, the treatment and the follow-up. The data was retrospectively collected by a chart review, the gross photographs of the specimens and the MRI or CT-scan of the primary lesion. Chest CT, bone scanning and ultrasonography of the regional lymph nodes were performed to rule out metastatic disease. The patients with perioperative signs of regional and/or distant metastatic disease and dedifferentiated liposarcomas were excluded from this study. Eighty-five cases of liposarcomas were eligible for this study. All the cases had been followed-up for at least 3 years from the time of surgery (mean follow-up period: 60 months, range: 36-165 months) or until their death. All the patients had been operated on with a curative intent, and 22 of them had received adjuvant chemotherapy and 7 cases had received postoperative radiotherapy. The 85 patients included 39 men (46%) and 46 women (54%) with a mean age of 52 years (range: 14-86 years) at presentation to our institute. The primary tumors were located in the lower extremities (n=53), upper extremities (n=13), pelvic area (n=10) and the trunk or abdominal wall (n=9). The pathological diagnosis and histological grading were reevaluated by two of our experienced sarcoma pathologists, and they had no knowledge of the clinical course. The malignancy grading followed a four-grade scale, and staging followed the American Joint Committee on Cancer (AJCC) staging system as revised in 2002.⁴ The histological classification of liposarcoma was three main types: well differentiated (n=21), myxoid/round cell (n=34) and pleomorphic liposarcoma (n=30). Stage I was found in 30 cases, stage II was found in 15 cases and stage III was found in 40 cases. The myxoid/round cell liposarcomas were distributed from stage I to III, depending on the presence of necrosis, the mitotic rate, the round cell component and the tumor size. The times until regional/distant metastasis and local recurrence were recorded separately.

Tissue microarray and immunohistochemical analysis

The analyzed tissues were the excised liposarcoma specimens from 68 cases and the pre-chemotherapy incisional biopsy specimens from 17 cases. The paraffin blocks were reevaluated for selecting representative areas without spontaneous necrosis. For each case, all the histologically well-preserved tumor tissues were circled on the guide slides with using a colored marker. The formalin-fixed, paraffin-embedded tissues corresponding to the histological sections were sampled from these marked regions by using a specialized manual arraying instrument. Using this device, the marked slides were aligned with the original tissue block, and a cylindrical core (3 mm in diameter) was removed

from the original blocks. After the array was completed, the tissue microarray blocks were sectioned at 4- μ m thickness.

We performed immunohistochemical staining on the liposarcoma tissue. Paraffin sections from the placenta and normal breast tissues were used in parallel as positive and negative controls, respectively. By staining the control tissues, the optimal antibody dilution was found to be 1:100. The negative control was prepared by substituting non-immune serum for antibody. We used the Zymed non-biotin amplification system (Zymed, CA, USA). Briefly, the tissue sections were dewaxed in xylene for more than 20 min and they were sequentially hydrated in 100%, 95%, 90%, and 80% ethanol solutions. After rinsing with water for 5 min, the sections were then pretreated with 0.01 M sodium citrate buffer and autoclaved for 1 min at 121°C to retrieve the antigen.

After rinsing, the endogenous peroxidase activity was blocked by treatment with 3% H₂O₂ for 30 min. The primary mouse monoclonal antibody against ezrin (mouse monoclonal antibody, Santa Cruz, CA, USA) was applied to the sections overnight in a moist chamber at 4°C. After rinsing with PBS, the slides were incubated with secondary antibody for 10 min at room temperature and then they were rinsed with PBS. The sections were incubated in tertiary antibody-horseradish peroxidase (HRP) conjugate for 10 min, rinsed in PBS and incubated with diaminobenzidine for 10 min. After counterstaining with Meyer's hematoxylin, the slides were dehydrated and mounted.

Assessment of the immunohistochemical data

Ezrin immunoreactivity was detected in the membrane or cytoplasm of the tumor cells (Fig. 1). This immunoreactivity was interpreted in a semiquantitative manner by analyzing the extent of the staining positivity of the tumor cells. The interpretation score was as follows: 0, <10% tumor cell positivity; +1, 10% to 20% tumor cell positivity; +2, 20% to 50% tumor cell positivity, and +3, greater than 50% tumor cell positivity. The score 0 was regarded as negative, while the scores +1, +2 and +3 were regarded as positive on the statistical analysis. Two pathologists evaluated the staining independently, and any discrepancy was resolved by a consensus review.

Statistical analysis

The correlation between the ezrin expression and metastasis was analyzed by the Kaplan-Meier method, and the differences were calculated with using the log-rank test. Associations bet-

ween the ezrin expression and the other clinical variables were determined with using two-tailed Fisher's exact test.

Cox's proportional hazard regression model was used for multivariate analysis. All the calculations were done with SPSS 10.0 software and p-values <0.05 were accepted as significant.

RESULTS

Clinicopathological characteristics of liposarcoma

Overall, 27 (31.8%) of the 85 cases of liposarcomas showed distant metastasis. As the AJCC stage advanced, the rate of metastasis increased. The pleomorphic type showed the highest

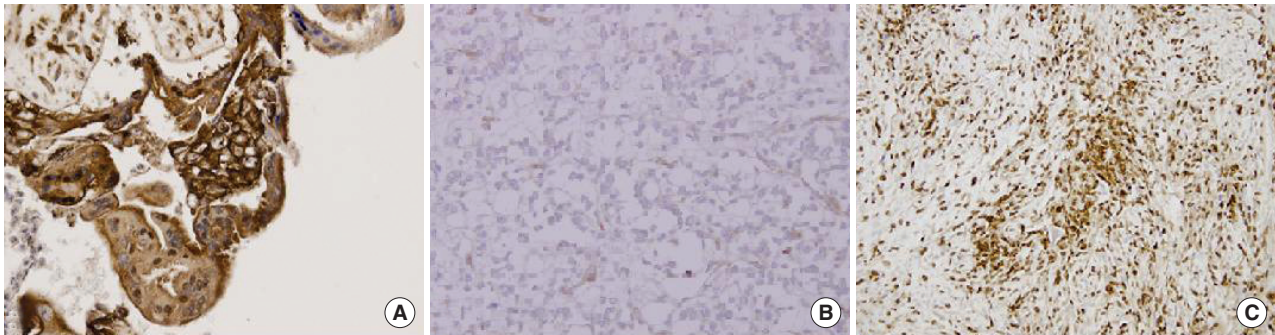


Fig. 1. These photomicrographs show membranous and cytoplasmic staining of ezrin in control placental tissue (A) and in representative liposarcoma samples with no ezrin expression (B) and strong ezrin expression (C).

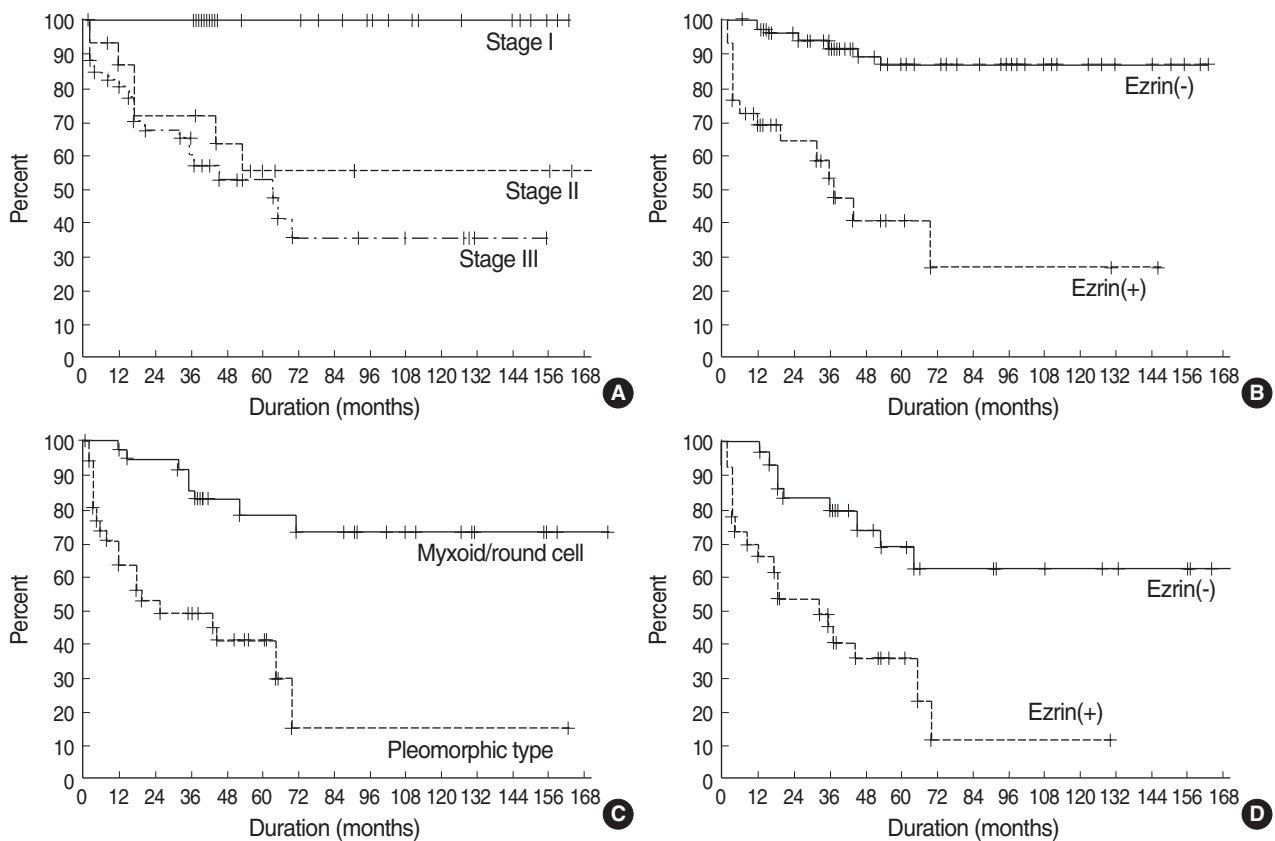


Fig. 2. The association of tumor stage (A), and ezrin expression (B) with metastasis is illustrated for 85 patients who had liposarcoma. The association of histologic subtype (C) and ezrin expression (D) with metastasis is illustrated for 55 patients who had high-grade liposarcoma.

metastatic rate (Table 1). The metastasis-free survival of stage I was 100% at 163 months, that of stage II was 55.8% at 176 months and that of stage III was 35.3% at 155 months (Fig. 2).

Twenty-two of the 55 cases of high grade liposarcomas showed local recurrence with an average of 1.9 events (range: 1-7 events), while 9 (36%) of the 25 cases of the high grade myxoid group and 13 (43%) of the 30 cases of the pleomorphic type showed local recurrence (Table 2).

Ezrin expression

Overall, 29 patients (34.1%) showed an ezrin expression. The rate of ezrin expression was as follows: 4.8% in the well-differentiated liposarcoma, 14.7% in the myxoid/round cell liposarcoma and 76.7% in the pleomorphic liposarcoma. For the myxoid/round cell liposarcoma, only the large tumor (>5 cm) showed an ezrin expression. The pleomorphic liposarcoma showed strong ezrin positivity, and this was independent of the size (Table 3). Table 4 provides a comparison of the clinicopathological factors

Table 1. Rate of systemic metastasis according to the stage and histologic subtype in 85 liposarcoma patients

	No. of patients (%)			
	Stage I	Stage II	Stage III	Total
Well-differentiated	0/21 (0)	-	-	0/21 (0)
Myxoid/round cell	0/9 (0)	2/6 (33.3)	6/19 (31)	8/34 (23.5)
Pleomorphic	-	4/9 (44)	15/21 (71)	19/30 (63.3)
Total	0/30 (0)	6/15 (40)	21/40 (52.5)	27/85 (31.8)

Table 2. Local recurrence rate according to the stage and histologic subtype in 85 liposarcoma patients

	No. of patients (%)			
	Stage I	Stage II	Stage III	Total
Well-differentiated	0/21 (0)	-	-	0/21 (0)
Myxoid/round cell	0/9 (0)	2/6 (33.3)	7/19 (36.8)	9/34 (26.5)
Pleomorphic	-	5/9 (55.5)	8/21 (38.1)	13/30 (43.3)
Total	0/30 (0)	7/15 (46.7)	15/40 (37.5)	22/85 (25.9)

Table 3. Ezrin expression rate according to the stage and histologic subtype in 85 liposarcoma patients

	No. of patients (%)			
	Stage I	Stage II	Stage III	Total
Well-differentiated	1/21 (4.8)	-	-	1/21 (4.8)
Myxoid/round cell	0/9 (0)	0/6 (0)	5/19 (26.3)	5/34 (14.7)
Pleomorphic	-	7/9 (77.8)	16/21 (76.1)	23/30 (76.7)
Total	1/30 (3.3)	7/15 (46.7)	21/40 (52.5)	29/85 (34.1)

between the patients who had tumors with negative or positive ezrin expressions. The table shows that the ezrin expression was significantly associated with the tumor location, the stage, the histologic subtype and the development of metastasis.

Prognostic factors for metastasis of high-grade liposarcoma

Because none of the patients with low-grade liposarcoma developed distant metastasis, we excluded them from the survival analysis. For the subset of the 55 cases of high-grade liposarcoma, survival analysis by the log-rank test revealed the histological subtype (p=0.004), and ezrin-positivity (p=0.0001) as significant prognostic factors (Fig. 2). On multivariate analyses, only the ezrin expression was shown to be an independent prognostic marker for the development of metastatic disease (p=0.008). Other clinicopathological factors such as gender, age,

Table 4. Comparison of clinicopathological factors by ezrin expression for 85 liposarcoma patients

	No. of patients (%)		p-value
	Ezrin (-)	Ezrin (+)	
Age at surgery			0.161
<50	25/33 (75.8)	8/33 (24.2)	
>50	31/52 (59.6)	21/52 (40.4)	
Gender			0.648
Male	27/39 (69.2)	12/39 (30.8)	
Female	29/46 (63)	17/46 (37)	
Location			0.001
Axial	6/19 (31.6)	13/19 (68.4)	
Extremity	50/66 (75.8)	16/66 (24.2)	
Size			0.789
<5 cm	12/19 (63.2)	7/19 (36.8)	
>5 cm	44/66 (66.7)	22/66 (33.3)	
Stage			<0.001
I	29/30 (96.7)	1/30 (3.3)	
II	8/15 (53.3)	7/15 (46.7)	
III	19/40 (47.5)	21/40 (52.5)	
Histologic subtype			<0.001
Well-differentiated	20/21 (95.2)	1/21 (4.8)	
Myxoid/round	29/34 (85.3)	5/34 (14.7)	
Pleomorphic	7/30 (23.3)	23/30 (76.7)	
Margin			0.357
Marginal/intralesional	34/48 (70.8)	14/48 (29.2)	
Wide	22/37 (59.5)	15/37 (40.5)	
Local recurrence			0.115
No	45/63 (71.4)	18/63 (28.6)	
Yes	11/22 (50)	11/22 (50)	
Metastasis			<0.001
No	49/58 (84.5)	9/58 (15.5)	
Yes	7/27 (25.9)	20/27 (74.1)	

Table 5. Clinicopathological characteristics of relapse pattern in 55 high-grade liposarcoma patients

	Ezrin		Subtype		Surgical margin		Average metastasis-free survival (months)
	-	+	m/r	pleo	w	m/i	
Metastasis (-) Recurrence (-)	15	7	14	8	11	11	51
Metastasis (+) Recurrence (-)	1	10	2	9	8	3	8
Metastasis (-) Recurrence (+)	5	1	3	3	1	5	66
Metastasis (+) Recurrence (+)	6	10	6	10	5	11	36

m/r, myxoid/round cell liposarcoma; pleo, pleomorphic liposarcoma; w, wide resection; m/i, marginal or intralesional resection.

the location of tumor, the size of tumor, the surgical margin, adjuvant chemotherapy and radiation therapy showed no correlation with metastasis. Overall, the rate of ezrin positivity was 74% in the metastatic cases (n=27). Those cases having metastasis within 1 year (n=10) showed a high ezrin expression (90%), while the cases having metastasis after 1 year (n=17) showed a relatively low rate of ezrin expression (64%).

Prognostic factors for local recurrence of high-grade liposarcoma

In the subset of the 55 cases of high-grade liposarcoma, univariate analysis revealed that a recurrent presentation (p=0.04) and the surgical margin (p=0.02) were meaningful prognostic factors for local recurrence. Multivariate analysis revealed both the surgical margin (p=0.01) and a recurrent presentation (p=0.02) as significant factors. Ezrin positivity did not correlate with local recurrence (p=0.35). Other clinicopathological factors such as gender, age, the location of tumor, the size of tumor, adjuvant chemotherapy and radiation therapy showed no correlation with local recurrence.

Clinicopathological characteristics of the relapse pattern of high-grade liposarcoma

Local recurrence and metastasis constituted the major treatment failures. According to local recurrence and metastasis, the 55 cases of high-grade liposarcomas could be divided into 4 groups (Table 5).

Metastasis (-) and recurrence (-)

There were 22 cases in this category. The ezrin expression rate was 31.8% and the myxoid/round cell type was 63.6%, and the average duration of follow up was 51 months (range: 35-176 months).

Metastasis (+) and recurrence (-)

There were 11 cases in this category. The ezrin expression rate was 90.9%, and the average duration to metastasis was 8 months (range: 2-32 months).

Metastasis (-) and recurrence (+)

There were 6 cases in this category. The ezrin expression rate was 17%, a marginal or intralesional margin was identified in 80%, and their average duration of follow up was 66 months (range: 39-163 month).

Metastasis (+) and recurrence (+)

There were 16 cases in this category. The ezrin expression rate was 62.5%, and a marginal or intralesional margin was identified in 68.8%. Their average duration of metastasis was 36 months (range: 2-70 month).

DISCUSSION

In the present study, we reconfirmed that the well-recognized prognostic variables such as the tumor stage and the histological type were valuable factors for predicting tumor behavior. Moreover, an ezrin expression was found to be an independent prognostic factor for metastasis-free survival for the patients with high-grade liposarcoma.

For the stage I liposarcoma, including the well-differentiated and low-grade myxoid/round cell liposarcoma, only one case showed an ezrin expression. Most of the stage I liposarcomas were located in the extremities. A low ezrin expression and the scarce axial location may have caused a significant association of the ezrin expression with the tumor location and stage. For the subset of 55 high-grade liposarcoma patients, there was no significant correlation of an ezrin expression with the tumors' location and stage.

For the myxoid/round cell liposarcoma, the exact evaluation of the tumor grade is the most important means to predict the clinical course.⁹ However, for the high-grade myxoid/round cell liposarcoma, predicting the clinical course by simply assessing all the known prognostic factors is difficult at best.⁵ An ezrin

expression was observed only in the advanced cases; nevertheless, no correlation was found between an ezrin expression and metastasis for the high-grade myxoid/round cell liposarcoma. This is most likely due to the fact that only a limited number of high-grade myxoid/round cell liposarcomas were examined in this study.

Pleomorphic liposarcoma showed the worst prognosis and the highest rate of an ezrin expression. Furthermore, an ezrin expression was frequently observed from the early stage of tumor progression. Considering the fact that the pleomorphic morphology means poor differentiation, an ezrin expression appears to be related to tumor progression. The significant role of ezrin in tumor progression has been established in an experimental setting, yet the clinical usefulness of this is currently under evaluation.⁷

A recent study on the prognostic impact of an ezrin expression in high-grade soft tissue sarcoma included malignant fibrous histiocytoma as the main pathologic type.⁸ Of the 7 cases of liposarcoma, one (14%) expressed ezrin, whereas all the 7 cases had metastasis at the final follow up. In our study, the overall ezrin expression rate of high-grade liposarcomas was 50.9%, which is much higher than that reported by the above study. Furthermore, the expression rate of ezrin was 20% and 76.6% in the high-grade myxoid/round cell type and the pleomorphic type, respectively. The difference in the expression rate can partly be explained by the different proportions of the histologic types in the two series.

The result of treatment failure of soft tissue sarcomas is local recurrence and distant metastasis.¹⁰ Local recurrence tends to be regarded as surgical failure due to the narrow width of the surgical margin, yet aggressive tumor biology may also have some impact on this. An inadequate surgical margin would be a predisposing condition of local recurrence, and the aggressiveness of tumor may play a triggering role. The difference between the width of the obtained gross surgical margin and that of the microscopic tumor infiltration may determine the final outcome of local recurrence. Aggressive tumor biology, and especially infiltration of tumor, has been suggested to be related with an ezrin expression.⁸ In our study, the fact that an ezrin expression had no correlation with local recurrence implies that the roles of the surgical margin and tumor aggressiveness are interrelated for local failure.

Metastasis is a highly complicated biological process. The group of patients who showed early metastasis without local recurrence exhibited the highest rate of an ezrin expression (90.9%), whereas the patients who had multiple local recurrences

without distant metastasis demonstrated the lowest rate of an ezrin expression (16.7%). These glaringly different figures reflect the significant role of ezrin played in distant metastasis. However, some cases showed an inverse correlation between ezrin positivity and metastasis, indicating that complex mechanisms other than an ezrin expression are involved in the metastatic process.

The roles of chemotherapy and radiation therapy for treating high-grade sarcoma remain a controversial issue.^{11,12} For high-grade liposarcoma, myxoid liposarcoma has been reported to be sensitive to chemotherapy.¹³ In the present study, chemotherapy and radiation therapy were found to play no significant role in the event-free survival of high-grade liposarcoma patients. New drugs, such as rapamycin, that inhibit ezrin-mediated metastasis should be considered as part of a chemotherapeutic regimen, and especially for the ezrin positive cases.¹⁴

In conclusion, an ezrin expression in liposarcoma seems to be associated with metastasis and it could be valuable as an additional prognostic marker for predicting the patient's outcome.

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