

Chronic Myeloproliferative Disorders

Altered apoptosis and cell cycling of mast cells in bone marrow lesions of patients with systemic mastocytosis

Enhanced expression of the apoptosis-preventing protein bcl-xL and the cell cycle-regulating protein p21 was observed in bone marrow infiltrates of systemic mastocytosis. Expression of bcl-2, Ki67, and p53 as well as ISEL apoptosis staining were comparable in patients with mastocytosis and in controls. An altered rate of apoptosis and cell cycling may contribute to accumulation of mast cells in mastocytosis.

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Systemic mastocytosis is characterized by accumulations of mast cells, particularly in bone marrow.¹ Adult mastocytosis is usually associated with gain-of-function mutations of *c-KIT*. Most patients show an indolent course (indolent systemic mastocytosis, ISM), however, there are also small subgroups affected by concomitant hematologic neoplasms (systemic mastocytosis with an associated hematologic disease, SM-AHNMD), an aggressive form or mast cell leukemia.² We have recently described that mast cells in cutaneous lesions of mastocytosis show a decreased rate of apoptosis associated with enhanced expression of bcl-2 and p53 proteins.^{3,4} Expression of bcl-xL and p21 (WAF1/CIP1) was not altered in skin lesions. In contrast, in a limited number of patients, we and others have observed enhanced levels of bcl-xL, but not bcl-2^{4,5} and p53,⁶ in bone marrow infiltrates of mastocytosis.

In order to extend our previous observations and to systematically analyze whether an altered rate of mast cell apoptosis or proliferation also contributes to accumulation of mast cells in bone marrow infiltrates, we studied the distribution of bcl-2, bcl-xL, p53, p21, and Ki67 protein by immunohistochemical staining of bone marrow sections of 14 patients with systemic mastocytosis (Table 1).^{3,4} Apoptotic cells were visualized applying the method of *in situ* end labeling (ISEL).⁷ Serial sections were cut and mast cells were identified immunohistochemically using a tryptase-specific monoclonal antibody.⁵ Out of the fourteen patients, twelve patients suffered from ISM and two patients from SM-AHNMD (the associated hematologic disease being acute myeloid leukemia in both cases). Patients with mastocytosis were compared to two control patients with a secondary increase of mast cells due to other diseases (reactive bone marrow, RBM). One of these patients suffered from large B-cell lymphoma without bone marrow infiltration, the other from polyneuropathy and weight loss without specific bone marrow pathology. The degree of staining was graded semiquantitatively using the following score: 0, mastocytosis: no reactivity of dense focal infiltrates containing mast cells, RBM: no reactivity of mast cells; 1, mastocytosis: reactivity in <30% of infiltrates, RBM: reactivity in <30% of mast cells; 2, reactivity in 30% to 70% of infiltrates or mast cells; 3, reactivity in >70% of infiltrates or mast cells. Confirming our previous studies,⁴ patients with

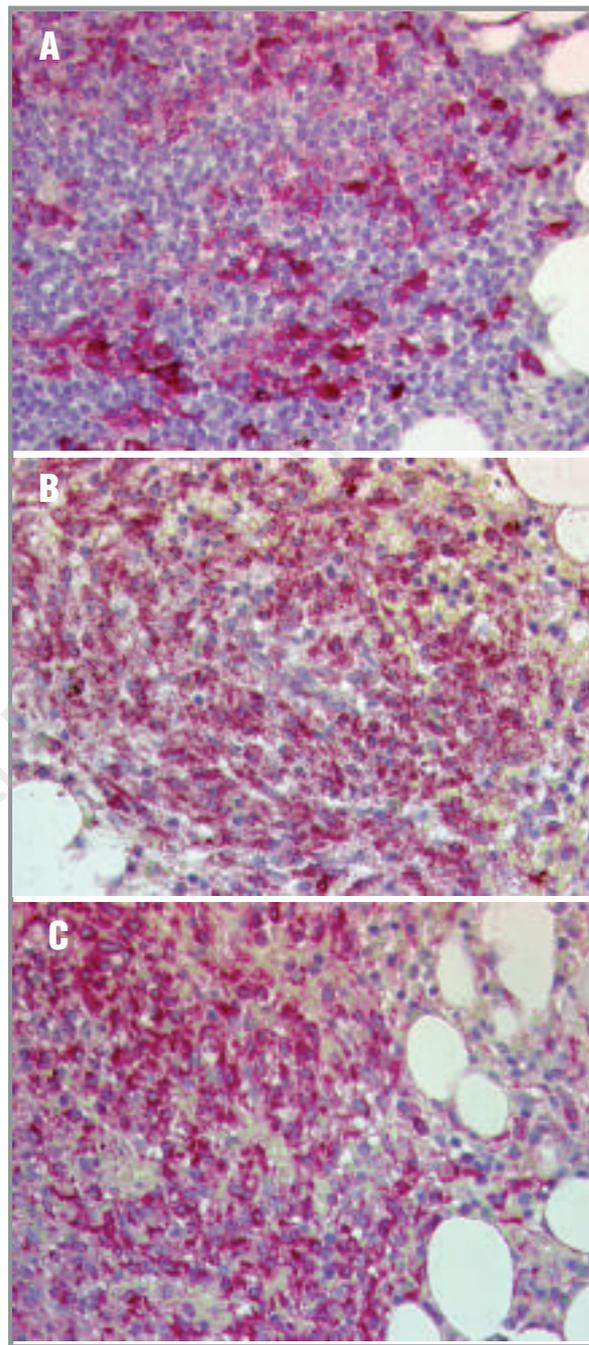


Figure 1. Mast cells in the bone marrow of patients with mastocytosis were visualized immunohistochemically by tryptase staining (A). Expression of bcl-xL protein in dense mast cell infiltrates of the bone marrow in ISM (B). Expression of p21 protein in ISM bone marrow (C). All photographs show immunohistochemical stainings of sections from patient #11. Original magnification $\times 250$.

Table 1. Degree of immunoreactivity for bcl-2, bcl-xL, p53, p21, Ki67, and ISEL apoptosis staining as determined by a semiquantitative score in mast cell infiltrates of the bone marrow in 12 patients with ISM, 2 patients with SM-AHNMD, and 2 control patients with RBM.

No.	Sex	Age (yrs)	Diagnosis	Mast cell infiltrates (%)	bcl-2	bcl-xL	p53	p21	Ki67	ISEL
01	M	38	RBM	0	0	0	0	0	0	0
02	F	60	RBM	0	0	0	0	0	0	0
03	M	45	ISM	20	0	3	1	1	NT	0
04	M	35	ISM	30	0	3	1	1	0	0
05	F	39	ISM	20	0	3	1	2	0	2
06	F	54	ISM	40	0	3	0	1	0	0
07	F	31	ISM	10	0	0	1	1	NT	1
08	F	45	ISM	5	1	3	1	1	0	0
09	M	48	ISM	5	0	1	1	1	0	0
10	M	57	ISM	5	0	3	1	2	0	1
11	F	31	ISM	5	0	3	1	1	0	2
12	F	65	ISM	20	0	3	1	1	2	1
13	M	72	ISM	15	0	1	0	1	1	1
14	F	54	ISM	70	0	3	1	2	0	1
15	M	71	SM-AHNMD	100	1	1	NT	NT	1	NT
16	M	60	SM-AHNMD	90	0	0	1	1	1	0
Mean score (ISM and SM-AHNMD cases)					0.14	2.14	0.85	1.23	0.42	0.62
Standard deviation					0.36	1.23	0.38	0.44	0.67	0.77
Standard error of the mean					0.09	0.32	0.10	0.12	0.18	0.20

mastocytosis showed significantly enhanced levels of bcl-xL expression (Figure 1B, Table 1), whereas expression of bcl-2 was absent or low in mastocytosis as well as controls. In contrast to the lack of expression of p21 in skin lesions,³ bone marrow infiltrates were also found to consistently express moderate levels of p21 (Figure 1C, Table 1), indicating that cycling of mast cells may additionally be altered in mastocytosis. Five out of thirteen patients showed a higher score for p21 than for p53. Reactivity for Ki67 and ISEL staining was low in most patients and not significantly higher than in the controls with RBM (Table 1).

Overexpression of bcl-xL leading to prolonged survival of cells has been demonstrated in a series of neoplastic diseases, including hematopoietic malignancies.⁸ *In vitro* studies have shown that activation of *KIT* induces expression of bcl-xL, possibly via induction of transcription factors such as *STAT3* (signal transducer and activator of transcription 3).^{4,8} Since adult mastocytosis is usually associated with activating *c-KIT* mutations, it is possible that these mutations are linked to bcl-xL overexpression, although increased bcl-xL expression was not observed in two patients with mast cell leukemia^{5,9} who are also known to express *c-KIT* mutations. In addition, cutaneous lesions in mastocytosis did not overexpress bcl-xL, but instead showed upregulation of bcl-2.⁴ Provided that mastocytosis is a clonal disease, it can be assumed that overexpression of bcl-xL in bone marrow lesions of ISM as well as enhanced bcl-2 expression in cutaneous lesions represent secondary alterations following a more complex activation of cells in their respective tissue environments. Unlike most patients with ISM, the two patients in our series with SM-AHNMD failed to overexpress bcl-xL. On the other hand, Jordan *et al.* observed an increased expression of bcl-xL in two out of four patients

with SM-AHNMD.⁵ A similar process that leads to overexpression of bcl-x may underlie upregulation of p21 in bone marrow infiltrates. p21 is known to regulate cell cycle progression and increased levels of p21 have been demonstrated in various tumors. In hematopoietic progenitor cells, activation of *KIT* has been shown to induce expression of p21.¹⁰ However, comparable to bcl-xL expression, p21 was only upregulated in bone marrow lesions, but not in cutaneous infiltrates,³ suggesting that *KIT* activation is not the only mechanism that controls p21 expression in mastocytosis. The present data support the concept that alterations in mast cell apoptosis and cell cycling contribute to the pathogenesis of mastocytosis.

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Malignant Lymphomas

Primary non-Hodgkin's lymphoma of the breast: a report of 11 cases

The breast is a rare localization of primary non-Hodgkin's lymphoma. We review our 15 years' experience in 11 patients with primary breast lymphoma. Based on our experience and on literature data we support a management scheme with CHOP or CHOP-like combination chemotherapy followed by involved field radiotherapy.

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Primary breast lymphoma (PBL) is a rare disease and its prognosis and treatment have not been clearly defined. Relatively small cohorts of patients are reported in the literature.¹⁻⁴ A retrospective single Institution review was carried out on all lymphoma patients admitted to Cagliari Hematology Unit between January 1st, 1989 through December 31st, 2003 in order to select patients who had Ann-Arbor stage I-II E lymphoma of the breast.

Of 1293 consecutive cases of non-Hodgkin lymphoma, 11 cases (0.85%) fulfilling the Wiseman and Liao criteria for primary breast lymphoma were identified.⁵

The patients' characteristics are detailed in Table 1. The median age at presentation was 65 years (range 33-84 years). Stage was IE in 3 patients and IIE in 8, none presented with bilateral involvement. Histology revealed 8 cases of diffuse large B-cell lymphoma, one case of follicular grade 1 lymphoma and two cases of extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). None presented with B symptoms. All

patients were staged by total body computerized tomography and bone marrow biopsy. Gallium scanning was performed in 4 patients and lymphangiography in one.

Four patients had mastectomy and three a wide excision of the breast lesion (2 quadrantectomy and one tumorectomy); the other patients underwent diagnostic biopsy. Axillary dissection was performed in 2 patients.

Two patients (both with stage IE disease) were treated with surgery alone. The first (case 6) underwent a mastectomy for a clinical diagnosis of ductal carcinoma and, after the correct diagnosis, refused further treatment. The second patient (case 10) received no further treatment because of age and co-morbid conditions. Both patients are alive and free of disease 41 and 9 months, respectively, after diagnosis. Postoperative treatment of all but two patients included CHOP or CHOP-like chemotherapy. In the case with follicular lymphoma, the CHOP chemotherapy was combined with rituximab (CHOP-R). The other treatment schemes are given in Table 1.

Six patients received radiotherapy with the photon beam of a 6MV linear accelerator. The radiation dose delivered ranged from 36 Gy to 50 Gy directed to the breast alone (two cases), axilla alone (one case), breast and regional nodes (three cases). Conventional fractionation (1.8 - 2 Gy, five times a week) was used. All patients achieved a complete response by the end of the therapy. Ten patients are currently alive and free of disease with follow-up ranging from 9 to 123 months (median, 25 months).

Patient 4 was diagnosed as having stage IIE large B-cell PBL when she was 12 weeks pregnant. She refused pregnancy termination and therapy. Delivery was induced at the 35th week. Subsequently, after complete re-staging which confirmed PBL, she was treated with MACOP-B and 50 Gy radiotherapy to the involved field. She achieved a complete remission but systemic relapse occurred 4 months later and she subsequently died of progressive disease.

PBL is a rare, potentially curable, disease and has been considered a distinct clinicopathologic entity.⁶

The prognosis, as reported in the literature, varies as do the applied treatment modalities, which include surgery, radiotherapy and chemotherapy used alone or in combination. Mastectomy or wide excision, used in seven of our patients is no longer indicated and can be avoided.⁷ Radiotherapy has been advocated by many authors as the first-line treatment for stage I disease;⁸ the efficacy of this management in terms of local control was confirmed in our series since no patients treated with 36-50 Gy radiotherapy relapsed locally or in the regional nodes.

With a follow-up ranging from 9 to 130 months, ten out of eleven of our patients are alive and free of disease. Nine of them received anthracycline-based chemotherapy followed by radiotherapy in six.

Distant recurrence is reported to be a significant problem for patients with PBL with a high incidence of central nervous system relapses after CHOP or CHOP-like regimens in patients with aggressive lymphoma.^{1,9} Our study shows that favorable results can be obtained in well-staged primary breast lymphoma using anthracycline-based chemotherapy combined with local-regional radiotherapy. On the basis of our experience and literature data we treat PBL with 3-6 cycles of the CHOP (or CHOP-like) scheme according to the IPI score (3 cycles with an IPI score of nil); rituximab is added in cases of CD20 positive lymphomas. Our good results without any central nervous system prophylaxis in patients without risk factors suggest that central nervous system prophylaxis is not necessary in all patients with primary breast lymphoma.