Mast Cells in Squamous Cell Esophageal Carcinoma and Clinical Parameters

BEATRICE TINGE, DANIEL MOLIN, MICHAEL BERGQVIST, SIMON EKMAN and STEFAN BERGSTRÖM

Department of Oncology, Uppsala University Hospital, Uppsala SE-751 85, Sweden

Abstract. *Background: Esophageal carcinoma is a malignancy with a poor prognosis and new treatment modalities must be sought. One possibility that has been tested in patients with malignant melanoma is treatment which aims towards boosting the immune system. In the present study, we investigated the role of mast cells in patients with esophageal carcinoma. The intention was to determine whether a higher number of mast cells is associated with better survival and may thus be a marker for future immunotherapeutic studies. Materials and Methods: A total of 61 archived tumor samples were retrieved of patients having received treatment due to esophageal carcinoma at the Department of Oncology, Uppsala University Hospital, Sweden. The tissue specimens were fixed in formalin, embedded in paraffin and sectioned in 3 μm-thick sections. The monoclonal antibody G3, recognizing the mast cell-specific protein tryptase was used, and the avidin–biotin–horseradish peroxidase complex (ABC/HRP) and diaminobenzidine (DAB) techniques were used to visualisze tryptase-positive cells. The positive cells were counted in 10 randomly selected high power fields. Results: When the number of mast cells was investigated in conjunction with relapse, no correlation was found (p=0.38). The number of mast cells was also not associated with survival (p=0.96). Using a pre-defined cut-off value of 31, no significant changes in survival was found (p=0.79) between patients with mast cell numbers above this value compared to those with numbers below. Conclusion: We conclude that mast cells do not seem to be related to prognosis in patients with esophageal carcinoma.*

Esophageal carcinoma is the seventh most common cause of cancer-related death in the Western World and the incidence of esophageal adenocarcinoma is increasing (1).

Correspondence to: Michael Bergqvist, MD, Ph.D., Associate Professor, Department of Oncology, Uppsala University Hospital,751 85 Uppsala, Sweden. Tel: +46 186115634 Fax: +46 186115528, e-mail: Michael.Bergqvist@onkologi.uu.se

Key Words: Mast cells, esophageal carcinoma, survival.

The major challenge in the treatment of esophageal carcinoma is to reduce the risk of local recurrence. However, within the first year after surgery (or curatively intended radiation therapy), the majority of these patients relapse and the median survival is dismal (2, 3). Since currently available treatments seldom cure these patients, new treatment modalities must be sought for. One possibility that has been tested in patients with malignant melanoma is treatment which aims towards boosting the immune system (4). One of the major players in the immune system is the mast cell (5) Several studies have been published in which the role of mast cells in different malignancies was studied, as well as their role in angiogenesis. Mast cells have been shown to have antitumor functions (6, 7) and Henderson *et al.* demonstrated that mast cells are involved in the peroxidase system, the biological products of which are able to function as antitumor compounds (8, 9). In the present study, the role of mast cells was investigated in patients with squamous cell carcinoma as a pilot study of whether there seems to be a correlation with survival or clinical parameters.

Materials and Methods

Between 1990 and 2000, 126 patients were recorded as having received treatment due to esophageal carcinoma at the Department of Oncology, Uppsala University Hospital, Sweden. from these patients, a total of 61 tumor samples were retrieved and were investigated for the analysis of mast cells. The excluded patients did not have sufficient tumor material for the present analysis.

The following clinical parameters were recorded: age; gender; localization, grouped into an upper (15-24 cm), a middle (25-34 cm) and a lower (35-46 cm) part of the oesophagus; performance status at first admittance; smoking; histology and tumor stage at first admittance defined as localized or metastatic disease.

Immunohistochemistry. The tissue specimens were fixed in formalin, embedded in paraffin and sectioned in 3 μm-thick sections. The monoclonal antibody G3, recognizing the mast cell-specific protein tryptase was provided by Dr L. B. Schwartz, MCV, Richmond, VA, USA (10). The avidin–biotin–horseradish peroxidase complex (ABC/HRP) and diaminobenzidine (DAB) techniques were used to visualise the tryptase-positive cells. The positive cells were counted in 10 randomly selected high power fields under light microscopy by one author (Figure 2).

Table I. *Clinical characteristics of the included patients as well as the median number of mast cells and univariate analysis of investigated clinical parameters.*

Variable	Number of patients	Median survival (days)	Median number of mast cells	*p*-Value (significance of the variable as related to survival of the patients)
Gender				0.10
Male	42	224	26	
Female	19	368	26	
Age (years)				0.41
≤65	22	232	30	
>65	39	233	26	
Tumor stage				0.00001
Advanced disease	17	140	25	
Localized disease	44	368	30	
Tumor differentiation				0.9
Poor	13	249	26	
Medium	14	224	20	
Well	0	0	0	
Localization of tumour				0.15
Upper	8	217	47	
Middle	22	319	26	
Lower	27	224	25	
Performance status				0.000002
0	22	455	27	
1	30	228	32	
2	6	70	18	
3	3	23	20	
Weight change				0.35
Weight loss	50	225	26	
No weight loss	2	0	17	
Smoking habits				0.01
Non smoker	10	309	53	
Smoker	31	227	26	
Not determined	10	110	26	
Alcohol usage				0.97
Alcohol use	4	177	28	
No alcohol use	33	233	26	

Statistics. The survival functions were estimated with the Kaplan-Meier product limit method and the median survival time estimated with linear interpolation of the survival function. The log-rank test was used to compare survival in two groups. The backward stepwise logistic regression method was utilized for univariate statistical interpretation of the data. Cox regression was utilized for the multivariate analysis. Throughout this study, a 5% significance level was used in the statistical tests.

Results

Tumors from a total of 61 patients with squamous cell carcinoma of the esophagus were investigated for the presence of mast cells. Patient demographics and statistical analysis concerning differences between median mast cell counts and clinical variables are shown in Table I. Mast cells were detected in all patients. Table II shows the correlation analysis in which the mast cells have been investigated in conjunction with clinical parameters. When the number of mast cells were investigated in conjunction to relapse, no correlation was found (p=0.38). The number of mast cells was not associated with survival (p=0.96). Using a pre-defined cut-off value of 31 (as decided by the authors based on clinical observations from other solid tumors, data not published), no significant changes in survival was found (p=0.79) (Figure 1).

Table II. *Correlation analysis investigating the role of mast cell-numbers and clinical parameters in patients with esophageal cancer.*

	Valid	Spearman (R-value)	*p*-Value
Gender	61	–0.03	0.82
Tumor stage	61	0.19	0.15
Tumor differentiation	27	0.04	0.82
Localisation of tumor	57	–0.04	0.74
Performance status	61	–0.04	0.78
Smoking habit	51	–0.19	0.18
Alcohol usage	37	0.13	0.45
Weight	52	–0.18	0.20
Age	61	–0.09	0.51

Discussion

In the present study, we report that mast cells do not seem to be associated with prognosis in patients with esophageal carcinoma. We were unable to distinguish any clinical correlation of mast cells in the tumors, contradicting the role of modulating the immune system in patients with esophageal carcinoma. Patients with esophageal carcinoma are in general subjected to preoperative chemoradiotherapy followed by surgery, or in inoperable cases, curatively intended chemoradiotherapy. Despite these intense treatment modalities, survival rates are poor and this has increased the awareness of other available treatment modalities for this patient category. Immune-modulating treatments have much interest for patients with malignant melanoma and there are at least three approaches to boost antitumor CD8 T-cell responses in these patients (5). In patients with esophageal carcinoma, Toh *et al*. (11) injected autologous tumor-activated lymphocytes into patients with distant metastases from esophageal carcinoma, with clinically beneficially results. However, the numbers of studies in this field are limited for esophageal carcinoma patients. One of the major players in the immune system is the mast cell and this cell-type has been shown to regulate different functions in the inflammatory process (12) in which they seem to regulate, amongst other things, vascular functions (13). When the mast cells are stimulated, they release the

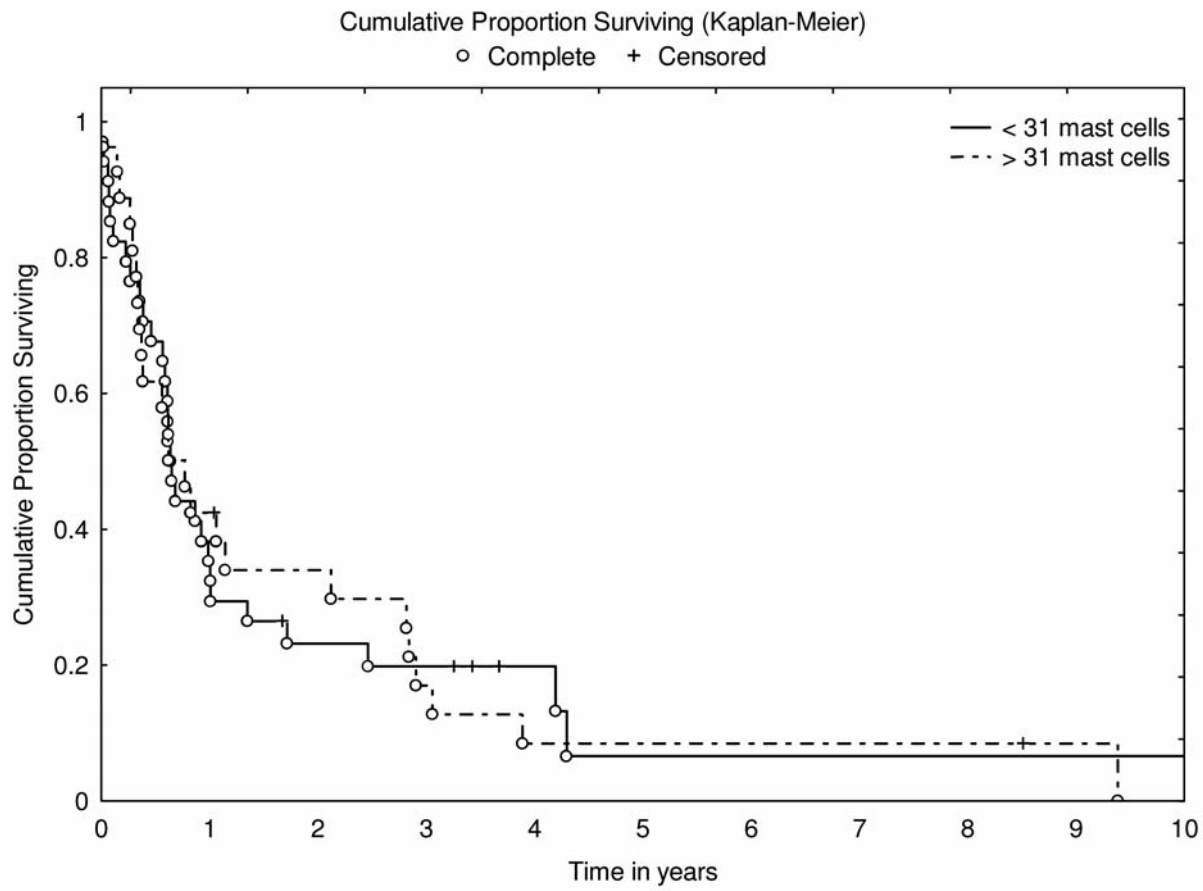

Figure 1. *Survival analysis for all patients based on a cut-off of 31 mast cells.*

contents of their granules, thereby activating leukocytes (14). Since tumor cells are surrounded by inflammatory cells, the role of the mast cells has gained an increasing interest in the field of tumor biology (15) and the mast cell has emerged as candidate for mediating tumor promotion (16). The issue of vascular invasion has been studied by several authors and it has been shown that mast cell granules produce or attract, amongst other things, histamine (17), basic fibroblast growth factor (18) and heparin (19). All these factors have been attributed to the promoting of tumor angiogenesis and, furthermore, mast cell tryptase was recently found to be a potent angiogenic factor, thus confirming these results (20). Moreover, in lymphomas, mast cells are suggested to cooperate in the induction of angiogenesis associated with disease progression (21). Antitumor effects have been reported in studies on breast cancer (22) as well as colorectal neoplasias (23) . Concerning esophageal carcinoma, Tomita *et al*. (24) showed that in their material from 48 patients with squamous cell carcinoma of the esophagus, there was a significant correlation between mast cell count and microvessel density. These studies suggest that mast cells might release substances intervening with and possibly affecting the tumor cell, thus causing tumor death. Other studies, however, have shown that mast cells enhance tumor proliferation *in vitro* (25, 26). In the present work, the number of mast cells within the primary tumor was not associated with survival (p=0.96). Nor was there any correlation with relapse (p=0.36). To further diminish the role of mast cells, no clinical correlation was found (Table II). In a study by Elpek *et al*., 53 patients with squamous cell carcinoma of the esophagus were investigated regarding correlation of mast cells and angiogenesis. The authors concluded that a high number of mast cells in conjunction with increased angiogenesis is associated with poorer prognosis (27). The role of mast cells is not fully conclusive for patients with esophageal carcinoma. In conclusion, in the present study, to our knowledge the largest in which the role of mast cells has been investigated in patients with esophageal carcinoma, we did not find any clinical correlations or associations of mast cell numbers with relapse or survival, implicating a limited role of the immune system in this tumor type, possibly negating there being a future role of immunotherapy for these patients.

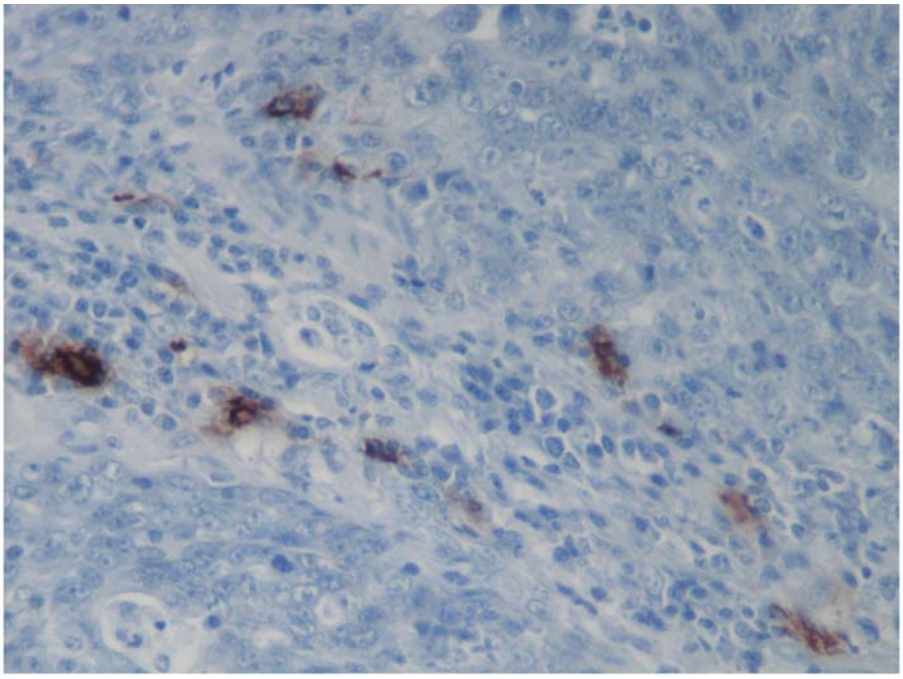

Figure 2. *Immunohistochemical staining of mast cells in a patient with esophageal carcinoma.*

Acknowledgements

The Authors are in debt to the Foundations Stiftelsen vid Onkologiska klinikens forskningsfond and Stiftelsen Gävle Cancerfond for providing funds for the present study. Furthermore, the Authors would like to express their gratitude to Martin Dreilich MD, Ph.D., Micael Strandell MD, as well as Rose-Marie Amini MD, Ph.D., for assistance in the evaluation of the clinical charts, as well as in the evaluation of the mast cells. The Authors also express their gratitude to Patrik Hesselius Ph.D. for statistical guidance.

References

1 Devesa SS, Blot WJ and Fraumeni JF Jr: Changing patterns in the incidence of esophageal andgastric carcinoma in the united states. Cancer *83*: 2049-2053, 1998.
2 Law SY, Fok M and Wong J: Pattern of recurrence after oesophageal resection for cancer: Clinical implications. Br J Surg *83*: 107-111, 1996.
3 John MJ, Flam MS, Mowry PA, Podolsky WJ, Xavier AM, Wittlinger PS and Padmanabhan A: Radiotherapy alone and chemoradiation for nonmetastatic esophageal carcinoma. A critical review of chemoradiation. Cancer *63*: 2397-2403, 1989.
4 Fang L, Lonsdorf AS and Hwang ST: Immunotherapy for advanced melanoma. J Invest Dermatol *128*: 2596-2605, 2008.
5 Rao KN and Brown MA: Mast cells: Multifaceted immune cells with diverse roles in health and disease. Ann NY Acad Sci *1143*: 83-104, 2008.
6 Ghiara P, Boraschi D, Villa L, Scapigliati G, Taddei C and Tagliabue A: *In vitro* generated mast cells express natural cytotoxicity against tumour cells. Immunology *55*: 317-324, 1985.
7 Farram E and Nelson DS: Mouse mast cells as antitumor effector cells. Cell Immunol *55*: 294-301, 1980.
8 Henderson WR, Chi EY, Jong EC and Klebanoff SJ: Mast cell-mediated tumor-cell cytotoxicity. Role of the peroxidase system. J Exp Med 153: 520-533, 1981.
9 Fisher ER and Fisher B: Role of mast cells in tumor growth. Arch Pathol *79*: 185-191, 1965.
10 Irani AM, Bradford TR, Kepley CL, Schechter NM and Schwartz LB: Detection of mct and mctc types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. J Histochem Cytochem *37*: 1509-1515, 1989.
11 Toh U, Sudo T, Kido K, Matono S, Sasahara H, Mine T, Tanaka T, Sueyoshi S, Fujita H, Shirouzu K and Yamana H: Locoregional adoptive immunotherapy resulted in regression in distant metastases of a recurrent esophageal cancer. Int J Clin Oncol *7*: 372-375. 2002.
12 Galli SJ and Wershil BK: The two faces of the mast cell. Nature 381:21-22, 1996.
13 Metcalfe DD, Baram D and Mekori YA: Mast cells. Physiol Rev *77*: 1033-1079, 1997.
14 Zhang Y, Ramos BF and Jakschik BA: Mast cells enhance the antibody-mediated injury of skin basement membrane in mice. J Immunol *149*: 2482-2487, 1992.
15 Takanami I, Takeuchi K and Naruke M: Mast cell density is associated with angiogenesis and poor prognosis in pulmonary adenocarcinoma. Cancer *88*: 2686-2692, 2000.

16 Norrby K: Mast cells and angiogenesis. APMIS *110*: 355-371, 2002.
17 Meininger CJ: Mast cells and tumor-associated angiogenesis. Chem Immunol *62*: 239-257, 1995.
18 Qu Z, Liebler JM, Powers MR, Galey T, Ahmadi P, Huang XN, Ansel JC, Butterfield JH, Planck SR and Rosenbaum JT: Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. Am J Pathol *147*: 564-573, 1995.
19 Azizkhan RG, Azizkhan JC, Zetter BR and Folkman J: Mast cell heparin stimulates migration of capillary endothelial cells *in vitro*. J Exp Med *152*: 931-944,1980.
20 Blair RJ, Meng H, Marchese MJ, Ren S, Schwartz LB, Tonnesen MG and Gruber BL: Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. J Clin Invest *99*: 2691-2700, 1997.
21 Ribatti D, Vacca A, Marzullo A, Nico B, Ria R, Roncali L and Dammacco F: Angiogenesis and mast cell density with tryptase activity increase simultaneously with pathological progression in B-cell non-Hodgkin's lymphomas. Int J Cancer *85*: 171-175, 2000.
22 Aaltomaa S, Lipponen P, Papinaho S and Kosma VM: Mast cells in breast cancer. Anticancer Res *13*: 785-788, 1993.
23 Fisher ER, Paik SM, Rockette H, Jones J, Caplan R and Fisher B: Prognostic significance of eosinophils and mast cells in rectal cancer: Findings from the national surgical adjuvant breast and bowel project (protocol r-01). Hum Pathol *20*: 159-163, 1989.
24 Tomita M, Matsuzaki Y, Edagawa M, Shimizu T, Hara M, Sekiya R and Onitsuka T: Association of mast cells with tumor angiogenesis in esophageal squamous cell carcinoma. Dis Esophagus *14*: 135-138, 2001.
25 Ju MJ, Qiu SJ, Gao Q, Fan J, Cai MY, Li YW and Tang ZY: Combination of peritumoral mast cells and T-regulatory cells predicts prognosis of hepatocellular carcinoma. Cancer Sci *100*: 1267-1274, 2009.
26 Molin D, Edstrom A, Glimelius I, Glimelius B, Nilsson G, Sundstrom C and Enblad G: Mast cell infiltration correlates with poor prognosis in Hodgkin's lymphoma. Br J Haematol *119*: 122-124, 2002.
27 Elpek GO, Gelen T, Aksoy NH, Erdogan A, Dertsiz L, Demircan A and Keles N: The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. J Clin Pathol *54*: 940-944, 2001.

Received October 30, 2009
Accepted December 14, 2009