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ARTICLE

Retrospective Analysis of the Prognostic Role of Tissue Eosinophil and Mast Cells in Hodgkin's Lymphoma

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The composition of reactive cell populations, which constitute the majority of tumor load in Hodgkin's lymphoma (HL), can influence the prognosis of the disease. Besides widely accepted and applied prognostic scores, the authors evaluate biological factors that may have a prognostic impact. Previous data indicate that the rate of eosinophils and mast cells in the reactive cell population, determined already at diagnosis, can be used for this purpose. Histological samples from 104 patients with HL with an average follow-up period of 110 (24-214) months were retrospectively analyzed. Mast cell positivity was associated with better overall survival, although this difference was only of borderline statistical significance (p=0.092). No significant difference was found in parameters like overall survival (OS, p=0.906) or event-free survival (EFS, p=0.307) of eosinophil-positive vs. -negative cases or in EFS (p=0.742) of mast cell-positive vs. -negative individuals (criterion for a positive specimen was more than 5% of appropriate cells in the reactive cell population). Looking at the effect of eosinophilia and mastocytosis together, there was no significant difference between the subgroups categorized according to the combined presence of the two cell types. It seems that tissue eosinophil and mast cell predominance have no prognostic value that could be used in clinical practice, although a tendency for correlation of mast cell positivity with overall survival could be seen. For a definitive statement, multicenter studies should be performed involving a higher number of patients suffering from HL. (Pathology Oncology Research Vol 13, No 3, 237–242)

Key words: Hodgkin's lymphoma, mast cell, eosinophil cell

Introduction

Unlike other hematologic and solid tumors, Hodgkin's lymphoma (HL) is characterized with a low (1-2%) frequency of malignant Hodgkin, Reed-Sternberg (HRS) cells and variants in the tumor tissue. The great majority of the tumor mass is composed by surrounding reactive cells (T and B lymphocytes, eosinophils, plasma cells, mastocytes and neutrophils) as well as stromal cells and connective tissue. By producing cytokines and chemokines, tumoral HRS cells, as neoplastic B cells, have an autocrine and paracrine influence on their environment while reactive cells also influence tumor cells. Besides other factors, e.g., Epstein-Barr virus (EBV) infection, these effects can also play a role in the escape of HRS cells from the control of the immune system. Under the influence of tumor necrosis factor-alpha produced by HRS cells, fibroblasts in the tumor microenvironment produce eotaxin, leading to eosinophil and T cell accumulation.[11] HRS cells express CCL28 and macrophage-derived chemokine (MDC), which may also play a role in eosinophil cell accumulation.[7,9] Eosinophil cells, at the same time, induce the proliferation of CD30- and CD40 antigen-positive HRS cells through their surface CD30 ligand (L) or CD40L, and increase fibroblast proliferation by producing transforming growth factor (TGF)-β1.[12,17,18] The production of CCL5 chemokine by HRS cells leads to mast cell accumulation in the tumor tissue.[5] Mast cells are CD30L-positive and activate HRS cells through CD30L-CD30 interaction.[14,15] All these effects seem to contribute to the survival of HRS cells and the subsistence of the tumor.

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Table 1. **Clinical characteristics of Hodgkin's lymphoma patients in relation to tissue eosinophilia and mastocytosis**

	Eosinophil– (n=40)	*Eosinophil+ (n=64)*	*Mastocyte– (n=26)*	*Mastocyte+ (n=78)*	*Eosinophil+ mastocyte+ (n=46)*	*Eosinophil– mastocyte– (n=8)*
Gender						
Female	17 (43%)	33 (52%)	11 (42%)	39 (50%)	25 (54%)	5 (63%)
Male	23 (57%)	31 (48%)	15 (58%)	39 (50%)	21 (46%)	3 (37%)
Histologic subtypes						
MC	18 (45%)	31 (48%)	11 (42%)	38 (49%)	23 (50%)	5 (63%)
NS	14 (35%)	21 (33%)	6 (23%)	29 (37%)	17 (37%)	0
LR	2 (5%)	1 (1%)	1 (4%)	2 (3%)	0	0
LD	2 (5%)	8 (13%)	5 (19%)	5 (6%)	4 (9%)	1 (12%)
NLP	1 (2%)	0	1 (4%)	0	0	1 (12%)
ND	3 (8%)	3 (5%)	2 (8%)	4 (5%)	2 (4%)	1 (12%)
Stage						
I-II	17 (46%)	30 (47%)	14 (54%)	33 (42%)	20 (43%)	4 (50%)
Favorable	6 (16%)	13 (20%)	5 (19%)	14 (18%)	8 (17%)	1 (13%)
Unfavorable	11 (30%)	17 (27%)	9 (35%)	19 (24%)	12 (26%)	3 (37%)
III-IV	23 (54%)	34 (53%)	12 (46%)	45 (58%)	26 (57%)	4 (50%)
IPS 0-3	18 (42%)	27 (42%)	10 (38%)	35 (45%)	21 (46%)	3 (37%)
IPS ≥4	5 (12%)	7 (11%)	2 (8%)	10 (13%)	5 (11%)	1 (13%)
General symptoms						
A	17 (43%)	35 (55%)	11 (42%)	42 (54%)	26 (57%)	3 (37%)
B	23 (57%)	29 (45%)	15 (58%)	36 (46%)	20 (43%)	5 (63%)
Bulky tumor	3 (8%)	12 (19%)	4 (15%)	11 (14%)	4 (9%)	1 (12%)

MC: mixed cellularity; NS: nodular sclerosis; LR: lymphocyte-rich; LD: lymphocyte-depleted; NLP: nodular lymphocyte-predominant; ND: not determined; A: absence of symptoms; B: presence of symptoms (unexplained fever, drenching night sweats, weight loss equal to 10% of the patient's weight)

New biological markers are being searched for in the literature to supplement existing markers used in clinical practice. By screening cases with good prognosis, it could be feasible to decrease the incidence of complications resulting from over-treatment, as well as the consequences of under-treatment in cases with bad prognosis. The prognostic investigation of HL tissue eosinophil and mast cell infiltration is a low-cost procedure and can be performed at the diagnosis of the disease. However, there is debate in the literature as to its importance, therefore, we decided to perform a retrospective study on the prognostic role of tissue eosinophilia and mastocytosis in our HL patients and, as a novelty in the relatively small amount of data in the literature, to study the effect of the combined presence of the two cell types.[1,3,13,14,26]

Materials and Methods

One hundred and four patients (50 females, 54 males; mean age at HL diagnosis: 33 (12-72) years, mean duration of HL: 110 (24-214) months) were randomly selected for the study of eosinophil and mast cell ratio in their histological samples. Tumor samples were taken at the diagnosis of the disease, prior to treatment. In each case, immunohistochemical revision of the samples based on the expression of CD15, CD20, CD30, CD45, EMA, BCL-6 and ALK1 was carried out at the Department of Pathology, using monoclonal antibodies, according to the WHO classification; histologic subtypes were designated by their English equivalents. The extension of HL was established by clinical examination and according to the Ann Arbor principles and their Cotswolds modifications. In early stage disease, the classification into groups of favorable and unfavorable prognosis was set up following the recommendations of the European Organization for the

Table 2. **Association of eosinophil and mast cell positivity with the presence of Epstein-Barr virus in histological samples of Hodgkin's lymphoma patients, based on LMP1-positivity**

Infiltration	*LMP1+ (n=26)*	*LMP1- (n=46)*
Eosinophil–	8 (31%)	18 (39%)
Eosinophil+	18 (69%)	28 (61%)
Mastocyte–	9 (35%)	8 (17%)
Mastocyte+	17 (65%)	38 (83%)

Research and Treatment of Cancer, while in advanced stages according to the International Prognostic Index (IPS) based on Hasenclever and Diehl's study.[8,10]

The ratio of eosinophilia was analyzed in paraffin-embedded, hematoxylin-eosin-stained samples in 5 randomly selected high-power fields (12.5x40) according to the technique described in the literature.[25] The quantity of eosinophil cells was given in the percentage of all cells seen in one field and this was averaged for the 5 fields. When the mean percentage of eosinophil cells in the reactive cells was <5%, the sample was considered negative, while eosinophilia was confirmed when the mean percentage of eosinophil cells was ≥5%.

The detection of mast cells was carried out in paraffin-embedded histological samples by immunohistochemistry using anti-tryptase monoclonal antibody (MAb AA1; DAKO, Glostrup, Denmark), according to the methodology described in the literature.[14] The incidence of mast cells was studied in 5 randomly selected high-power fields at the Department of Pathology. Similarly to the assessment of eosinophilia, tissue mastocytosis was considered in the case of a mast cell ratio ≥5%.

In 72 patients with HL, EBV association was detected in paraffin-embedded samples by immunohistochemical detection of latent membrane protein (LMP)1 in HRS cells using a mouse monoclonal antibody (DAKO).

For the study of overall survival (OS), the period between the diagnosis and death due to any reason was given. For event-free survival (EFS), the period between the first treatment and progression of the diseases, relapse or death due to any reason was given.

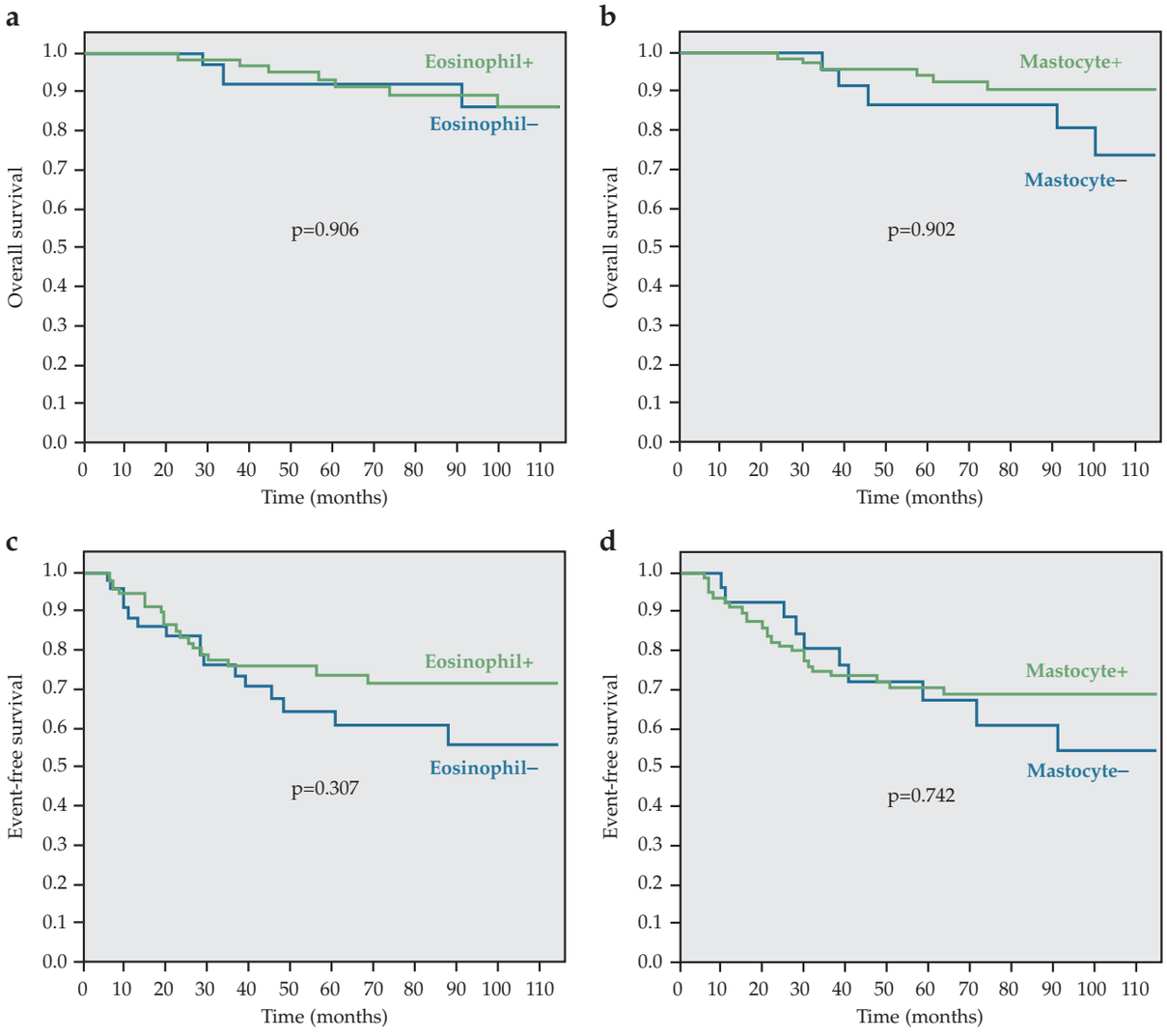


Figure 1. *Overall* ***(a, b)*** *and event-free* ***(c, d)*** *survival of Hodgkin's lymphoma patients in relation to eosinophil* ***(a, c)*** *and mast cell* ***(b, d)*** *infiltration*

Survival was analyzed by the Kaplan-Meier method. In the statistical analyses (χ^2, Fischer's exact test, log rank test) $p<0.05$ probability level was considered significant.

Results

Tissue eosinophilia and mastocytosis were found in 62% and 75% of the patients, respectively. The mean age of HL patients at diagnosis was 33 years. The mean age of patients with eosinophil positivity was 36 (12-72) years, and that of patients without infiltration was 31 (13-65) years. The mean age of patients with mast cell negativity was 30 (15-54) years, while that of patients with mast cell positivity was 34 (12-72) years; no significant differences were found.

A comparison of the clinical data of patients with eosinophil– and mast cell-positive and -negative tumors revealed no significant difference (*Table 1*). Similarly, no significant associations were found between EBV positivity and eosinophil/mast cell infiltration (*Table 2*).

Analyzing OS and EFS, no significant differences were found between patients with or without tissue eosinophilia or mastocytosis, although a trend of borderline significance (p=0.092) was observed for a more favorable OS in the case of patients with mast cell positivity (*Fig. 1*). The evaluation of the combined effect of tissue eosinophilia and mast cell infiltration revealed no significant differences in either OS or EFS between subgroups set up according to combinations of high or low numbers of eosinophils and mast cells (*Fig. 2*).

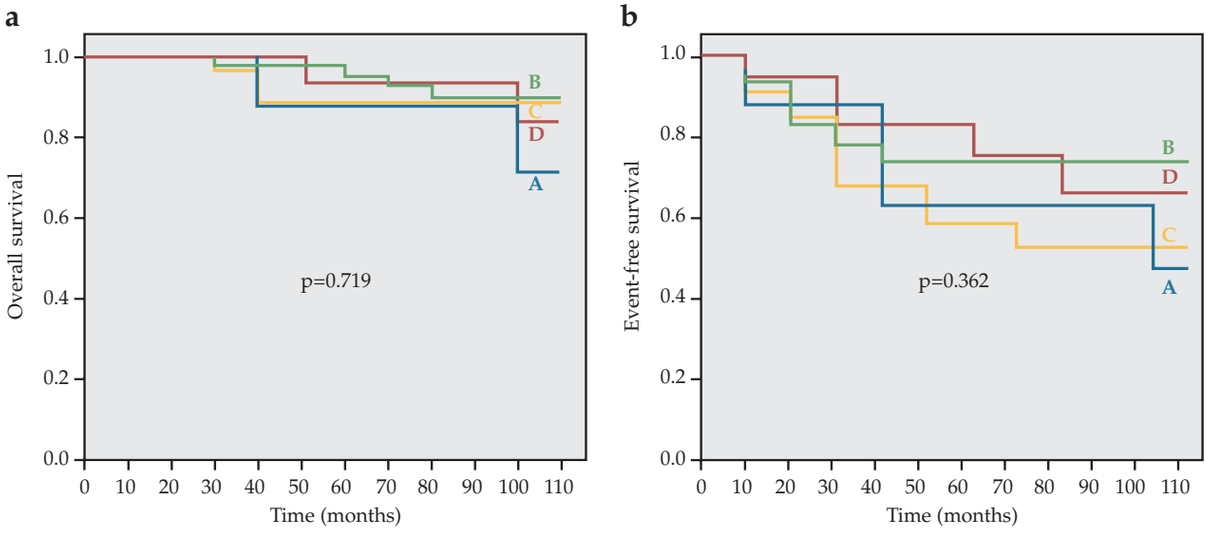
During the study, 14 of the 104 HL patients died. Four of the patients without tissue eosinophilia died, 2 of them due to the primary (HL) disease, 1 in pulmonary and 1 in colonic neoplasm. Of the patients with eosinophil-positive tumor 10 died, 7 due to the progression of the disease, 1 in lung, 1 in liver neoplasm and 1 in myocardial infarct. Of the patients without mast cells, 3 deaths were due to HL progression and 2 to pulmonary neoplasm, while of those with mast cell positivity, 6 deaths were due to the primary disease, 1 to liver, 1 to colonic neoplasm and 1 to myocardial infarct.

Based on the prognostic factors for OS and EFS used in clinical practice today we found significantly better OS in patients with favorable prognosis than in those with unfavorable prognosis (p=0.028), and significantly better EFS (p=0.013) and OS (p=0.008) parameters in the IPS 0-3 patient group compared to IPS 4 cases.

Discussion

A retrospective study was performed on the prevalence of eosinophil and mast cell infiltration in histological samples taken at HL diagnosis. Our results show that the ratio of both eosinophil and mast cell infiltration was similar in the mixed cellularity (MC) and nodular sclerosis (NS) histological subtypes; similarly to data in the literature,[21,26] the lowest ratio in classical HL was found in the lymphocyte-rich (LR) subtype, while in the nodular lymphocyte-predominant (NLP) one it did not occur at all.

Similarly to Axdorph et al.,[1] we did not find significant differences as regards to disease stage, general symptoms and gender in relation to tissue eosinophilia and mast cell infiltration. They, however, found a significantly higher



A: eosinophil–, mastocyte– (n=8), B: eosinophil+, mastocyte+ (n=46), C: eosinophil–, mastocyte+ (n=32), D: eosinophil+, mastocyte– (n=18)

Figure 2. *Overall* ***(a)*** *and event-free* ***(b)*** *survival of Hodgkin's lymphoma patients in relation to eosinophil and mast cell infiltration in combination*

prevalence of eosinophil infiltration in patients with bulky tumor, while we, similarly to von Wasielewski et al., did not find such a relationship.[1,26] The distribution of prognostic factors used in clinical practice did not show significant correlation with the incidence of tissue eosinophil and mast cell infiltration in our patients.

Through EBV infection, LMP1 appearing in HRS cells has an effect similar to that of tissue eosinophil cells; in *in vitro* and *in vivo* studies it has been shown to stimulate the growth of HRS cells and activate nuclear factor (NF)-κB and CD40.[16,22,26] Though their pathomechanism is the same as regards to tumor growth, the two factors cannot replace each other. Similarly to the results of Axdorph et al.,[1] we found that LMP1 positivity did not influence the appearance of eosinophilia in the tissues. Teruya-Feldstein et al. did not find any relationship between tissue eotaxin level and EBV infection either.[24]

Although not statistically significant, in our study OS was found better in the case of mastocytosis, while either OS or EFS of eosinophil-positive vs. eosinophil-negative cases, or EFS of mast cell-positive vs. mast cell-negative ones did not show significant difference. In contrast to our findings, Molin et al. observed that relapse-free survival was worse in patients with mast cell infiltration.[13,14] Glimelius et al. suggested that the less favorable survival rate found in cases with mast cell infiltration were not only due to NF-κB activation induced in HRS cells by mast cell CD30L positivity, but was also a consequence of an enhancement of tumor development by angiogenesis stimulation through increased IL-8 production.[6] Literature data confirm that both mast cell-derived heparin and histamine can be angiogenic.[19,23] In contrast, Samoszuk et al. found degranulated mast cells in peritumoral fibrotic tissues of human tumors, and showed the ability of heparin to inhibit the growth of primary and metastatic tumors.[20]

Similarly to our results, studying the role of eosinophil cell infiltration Axdorph et al. did not find significant differences as regards to EFS.[1] Several other studies, however, observed significantly worse treatment results, failure-free survival, EFS and OS in cases with tissue eosinophilia.[4,25,26]

To control our results, we also studied the patients' survival following the favorable-unfavorable and IPS classification used in clinical practice. We found significantly better OS in patients with favorable prognosis than in those with unfavorable prognosis and significantly better EFS and OS parameters in the IPS 0-3 group than in the IPS 4 one. We could not find such significant differences with regard to in eosinophil or mast cell infiltration.

In conclusion, in contrast to solid tumors where the appearance of tumor-infiltrating eosinophils predict better prognosis,[2] no significant differences were found in OS and EFS of HL patients with regard to eosinophil and mast cell infiltration, although a tendency for correlation with better OS was observed in the case of tissue mastocytosis. Data in the literature differ regarding the role of tissue eosinophilia, while studies on mast cell infiltration are scarce. Based on our experience, it seems that the prognostic strength of tissue eosinophilia or mastocytosis (or the two together) is not convincing enough for use in the clinical practice. To resolve this question, further studies involving a larger number of Hodgkin's lymphoma patients should be performed.

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