Tryptase- and chymase-positive mast cells as a possible prognostic factor in patients with Hodgkin's lymphoma

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Abstract: Objectives. The aim of the study was to identify potential prognostic factors in Hodgkin's lymphoma – HL patients by means of assessment of the influence of mast cells on clinical characteristics of the disease. **Patients adn methods.** Tryptase- and chymase- positive mast cell density was assessed in order to correlate it with histological type, staging, sex and age of patients. Tissue specimens were taken from a group of 72 patients treated for Hodgkin's lymphoma in the Department of Internal Medicine and Oncological Chemotherapy of the Silesian Medical Academy in Katowice from 1990 to 2002. The analyzed group consisted of 44 men (age 16–73 years, av. 39.2) and 28 women (age 15–73 years, av. 36.5) presenting 5 histological types of HL according to the WHO classification. Overall survival (OS) for the group ranged from 3 to 169 months (av. 64.5) while disease free survival (DFS) was from 4 to 167 months (av. 44.8). **Results.** The highest MCD-T and MCD-C was observed in NS while the lowest had characterized LD. A statistically significant difference in MCD-T was noted between NS and LD (p=0.0002). Despite the fact that increased MCD-T and MCD-C was observed in stage III of the disease and the lowest in stage IV. No correlation were found between MCD and stage, sex or age. Overall survival was assessed in correlation with the histological type and showed to be the best in MC and the worst in NS. **Conclusions.** Tryptase and chymase positive mast cell density is related to the histological type of HL. Increased mast cell density correlates with worse prognosis in patients with HL.

Keywords: chymase, Hodgkin's lymphoma, mast cells, prognosis, tryptase

INTRODUCTION

Hodgkin's lymphoma, also known as lymphogranulomatosis, was first described in 1832 by Thomas Hodgkin, who discovered general lymphadenopathy and splenomegaly in 7 autopsy cases suspecting their neoplastic origin [1]. In 1865 Wilkes and Greenfield described histiological lesions in that disease, pointing to chronic inflammatory infiltration of lymph nodes with a component of fibrosis and presence of polycariotic cells. Typical morphological features of malignant cells were published by Sternberg in 1889 and Reed in 1902. These authors described the cell size and morphology of their multilobar nuclei with prominent micronuclei. Presence of Hodgkin and Reed-Sternberg (HRS) cells became the basic diagnostic criterion of lymphogranulomatosis, today classified as Hodgkin's lymphoma (HL) [2,3]. According to the 1999 WHO classification Hodgkin's lymphoma is divided into 2 main histological types: LP (Nodular Lymphocyte Predominant Hodgkin Lymphoma – NLPHL) and Classical Hodgkin Lymphoma – CHL with the following subtypes: NS I, NS II – Nodular Sclerosis Classical Hodgkin Lymphoma – NSCHL, MC – Mixed Cellularity Classical Hodgkin Lymphoma – MCH, LR – Lymphocyte-rich Classical Hodgkin Lymphoma – LRCHL and LD – Lymphocyte-depleted Classical Hodgkin Lymphoma – LDCHL [4].

The prevalence of HL attains the value of 2–3.5 cases/100 000 per year that makes 8–12% of all lymphatic malignancies [5]. In spite of many long term multicenter studies the etiology of HL still remains unknown. Many facts point to the important role of infection as well as environmental and genetic factors [6,7,8]. Early studies suggested a multifocal origin of the disease but later it became clear that HL develops primarily in one focus, usually with nodal localization. Histological changes in HL are different than in other neoplasms due to their unique cellular composition: malignant HRS cells make uponly 0,1–2,0% of the total infiltration, while the rest is formed by benign reactive cells: lymphocytes, histiocytes,

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Table. Characteristics of 72 patients with Hodgkin's lymphoma

Histology	n	M	F	B-symptoms	Staging	Treatment Response	Death	MCD-T ±SEM	MCD-C ±SEM
LP	3	3	0	3	2 – III 1 – IV	PR-2 NR-1	3	8.1 ±3.4	2.8 ±0.2
NS	26	11	15	23	4 – II 11 – III 11 – IV	CR-6 PR-7 NR-13	15	10.2 ±2.8	5.6 ±2.2
MC	23	15	8	18	5 – II 14 – III 4 – IV	CR-15 PR-3 NR-5	6	7.9 ±2.7	3.9 ±1.8
LR	7	5	2	4	1 – I 3 – II 1 – III. 2 – IV	CR-4 PR-3	0	6.5 ±2.1	3.9 ±1.5
LD	13	10	3	11	1 – II 2 – III 10 – IV	CR-4 PR-1 NR-8	8	4.1 ±1.3	2.3 ±1.4

CR – complete remission, F – female, LD – lymphocyte depletion, LP – nodular lymphocyte predominant, LR – lymphocyte-rich, M – male, MC – mixed cellularity, MCD-C – chymase positive mast cell, NS – nodular sclerosis, NR – non remission, MCD-T – tryptase positive mast cell density, PR – partial remission

plasmocytes, fibroblast and mast cells [1]. Their percentage decreases with the progression of the disease in favor of malignant HRS cells.

Many studies have been performed to asses the relationship between histology of Hodgkin's lymphoma and clinical characteristics of the disease but the opinions about clinical validity of assessed parameters are ambiguous. The most important issue seems to be the discovery of the mechanisms of interaction between the elements of benign reactive infiltration and their influence on tumor metabolism. Recent papers describe the regulatory function of mast cells and the correlation between increased mast cell activity and the progression of disease. Suggested mechanism of this regulation could be the interaction between the CD-30 receptor of HRS cells and the CD-30L ligand characteristic for tryptase positive mast cells [9,10]. In vitro studies showed stimulation of HRS cells by MCs in the CD30 – CD30L reaction. This phenomenon might also be significant in vivo, but other mechanisms and mast cell related mediators are also plausible, for instance IL-9 or IL-13 [11].

The aim of this study was to asses the influence of tryptase- and chymase-positive mast cells on the clinical outcome in Hodgkin's lymphoma patients.

PATIENTS AND METHODS

The material consisted of paraffin fixed tissue specimens taken from 72 patients treated in the Oncological Chemotherapy Department of the Silesian Medical Academy in Katowice, Poland, from 1990 to 2002. The group consisted of 72 patients in ages from 15 to 73 (av. 36,5) years: 44 men and 28 women. The male/female ratio was 1,57:1. Clinical data was collected by reviewing the patient's charts and was part of the clinical database of the Oncological Chemotherapy Department of the Silesian Medical Academy. All cases were reclassified according to the updated WHO classification. Staging was based on Ann-Arbor criteria (modified by the Cotswold meeting). Treatment was either standard or consistent with the investigational protocols active during the time the patients were diagnosed.

The group was divided into 5 subgroups according to the histopathological subtype (WHO 1997 classification): LP – lymphocyte predominant (n = 3), NS-nodular sclerosis (n = 26), MC – mixed cellularity (n = 23), LR – lymphocyte rich (n = 7) and LD–lymphocyte depleted (n = 13). The characteristics of these 72 patients are seen in table. No allergies, autoaggresive diseases nor other proliferative diseases were found. Overall survival (OS) for the group was from 3 to 169 months (av. 64.5±), disease free survival (DFS) from 4 to 167 months respectively (av. 44.8±). 32 patients (44% of the group) died. Patients were treated according to the following treatment schedules: MOPP (mechlorethamine, vincristine, procarbazine, prednisone or COPP (cyclophosphamid, vincristine, procarbazine, prednisone) in 43 patients, MOPP/ABV (mechlorethamine, vincristine, procarbazine, prednisone /doxorubicin, bleomycin, vinblastine) or COPP/ABV (cyclophosphamide, vincristine, procarbazine, prednisone/doxorubi-

cin, bleomycin, vinblastine) in 24 patients and ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) in 5 patients. Complete remission (CR) was achieved in 29 (40.3%), partial remission (PR) in 16 (22.2%) and no remission (NR) in 27 (37.5%) patients.

IMMUNOHISTOCHEMISTRY

The paraffin embedded tissue specimens were sectioned in 3 μm thick sections. Monoclonal antibodies: Anti-Human--Mast Cell Tryptase and Anti-Human-Mast Cell Chymase (Novocastra Laboratories Ltd, Newcastle UK) were used for immunohistochemical staining. The avidin-biotin-horseradish peroxidase ABC kit (Novocastra Laboratories Ltd, Newcastle UK) and diaminobenzidine (DAB) techniques were used to visualize tryptase- and chymase-positive cells, then routine HE staining was performed. Specimens were then observed at 100x magnification in order to localize places of the highest MC concentration (light microscope Labophot, Nikon). Tryptase- and chymase-positive cells were then counted in 10 randomly selected high-power fields – area 0,152 mm^2 (HPF, 200x), by one investigator. The score from each 10 fields was averaged. Positive reaction for tryptase and chymase positive mastocytes was defined as evident granular cytoplasmatic reaction, according to manufacturer's specification.

STATISTICS

Statistical analysis was performed using Statistica® 6.0 software. The ANOVA, Kruskall-Wallis and Tukey RIR tests were used to compare MCD-T and MCD-C values between groups defined by histological subtype and staging. Kaplan-Meier method was used for survival analysis. Curves showing overall survival (OS) and disease free survival (DFS) according to MCD-T and MCD-C were constructed using a cutoff score of 10.0 for MCD-T and of 6.0 for MCD-C. Two curves were compared using Cox's F test. Overall survival was also assessed in relation to histological type. Curves were constructed for NS, MC and LD. LP and LR were not included because of an insufficient number of samples. Three curves were compared using Mantel procedure.

RESULTS

Mast cell density (MCD)

In affected lymph nodes the highest number of mast cells was observed around vessels, in collagen bands (NS type) and around necrotic foci. Tryptase and chymase positive mast cells showed a trend to form larger groups in NS type. The number of tryptase positive mast cells in field ranged from 2 to 64, for chymase positive MCs from 2 to 48 respectively. Main tryptase positive mast cell density (MCD-T) for the whole analyzed group was 7.9 ±0.8 and for main chymase positive mast cells (MCD-C) 4.2 ±0.5 respectively. The highest MCD-T was observed in NS type (10.2) (fig. 1.) while the lowest characterized LD (4.1) (fig. 2). MCD-C was highest in NS (5.6) and lowest in LD (2.3), as shown in table. Statistically significant difference in MCD-T was noted between NS and LD ($p = 0.0002$). The highest MCD-T and MCD-C was observed in stage III while the lowest was characteristic for stage IV but no statistically significant correlation was found between MCD, staging, age or sex.

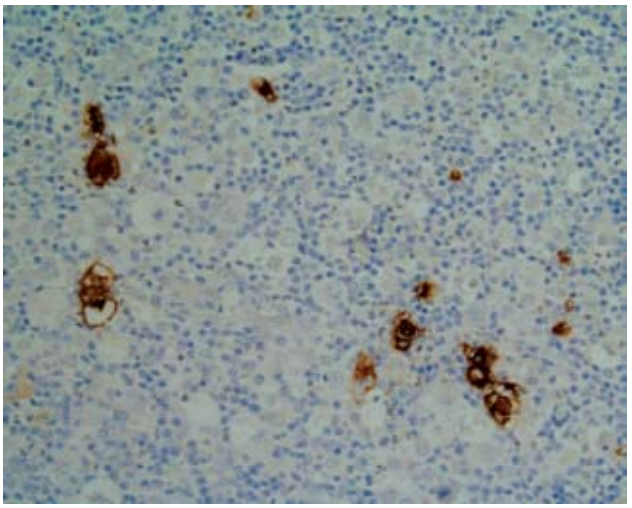

Fig. 1. Hodgkin lymphoma tissue – NS (nodular sclerosis) with the expression of tryptase in mast cells. Magnification: x200; mast cells shown in brown.

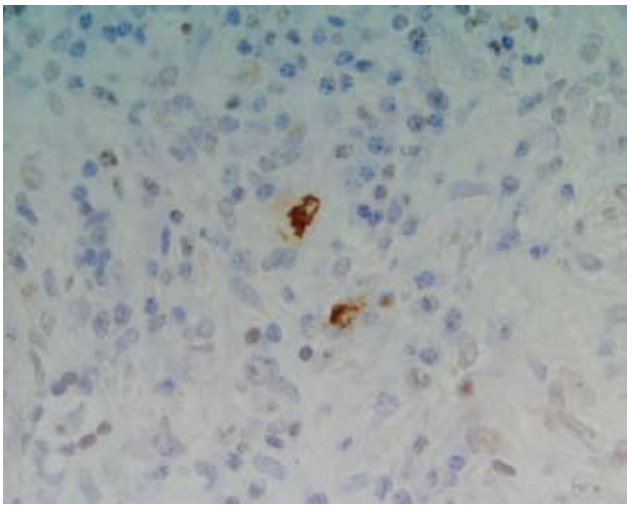

Fig. 2. Hodgkin lymphoma tissue – LD (lymphocyte depleted) with the expression of tryptase in mast cells. Magnification: x400; mast cells shown in brown.

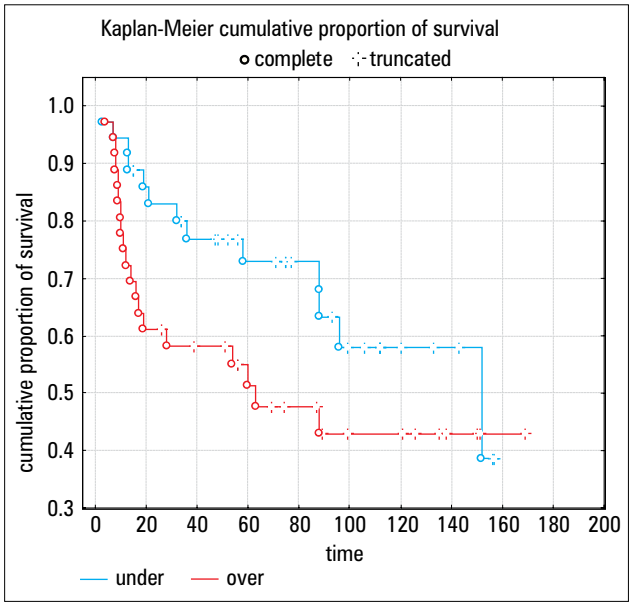

Fig. 3. Kaplan-Meier curves (DFS) for groups defined by MCD-T.

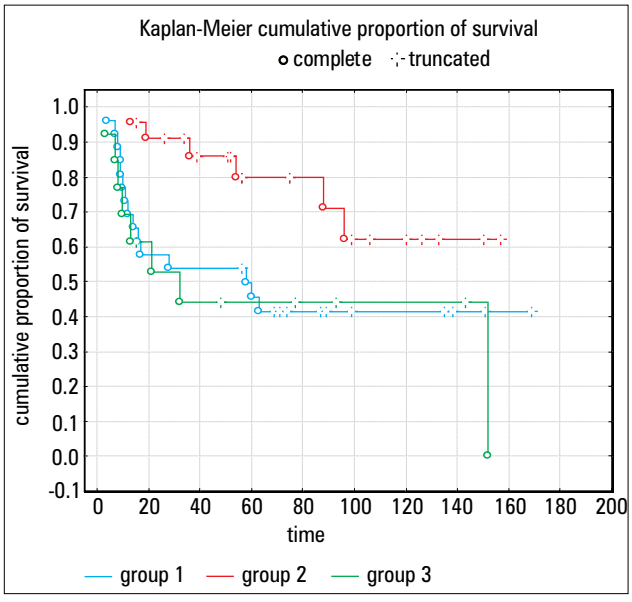

Fig. 4. Kaplan-Meier curves (OS) for types NS, MC, LD of Hodgkin's lymphoma.

Survival analysis

Statistical analysis revealed no significant correlation between overall survival (OS) and MCD-T ($p = 0.1$) or MCD-C ($p = 0.2$). The correlation between DFS and MCD-T showed a trend to statistical significance ($p = 0.06$) (fig. 3). No correlation was found between MCD-C and DFS ($p = 0.3$).

Overall survival according to histological type of Hodgkin's lymphoma was assessed for NS, MC and LD. Statistically significant difference was found between all 3 Kaplan-Meier curves ($p = 0.01$). The best overall survival was noted for MC type, significantly worse for NS (fig. 4). The frequency of death at early stage of the disease was similar for NS and LD types. Estimated 10-year survival was 40% for NS and 60% for MC (fig. 4).

DISCUSSION

Lately the usefulness of certain histomorphologic parameters as independent prognostic factors in Hodgkin's lymphoma has been widely discussed in the scientific journals [11,12,13]. Many papers in the last five years presented the relationships between affected lymph node histology, laboratory parameters and clinical characteristics [14,15]. Attempts have been made to assess the clinical validity of single histomorphologic parameters of both malignant and reactive cells [9,16,17] and to discover mechanisms of their influence on tumor metabolism.

Wide use of immunohistochemistry and molecular techniques facilitated the discovery of several autocrynic and paracrynic regulatory processes of interaction between HRS cells and benign elements of infiltration [9,10,18]. This feedback system is responsible for growth and differentiation of tumor cells as well as for local and remote tissue reactions. The possibility of molecular intervention at the level of these regulatory mechanisms could be a breakthrough in diagnostics and treatment of Hodgkin's lymphoma and other lymphoproliferative diseases.

Mast cells, especially the tryptase-positive subpopulation, seem to play an important role in Hodgkin's lymphoma biology [19]. Studies of Molin et al. from 2001 and 2002 [9,20] showed that mast cell infiltration correlates with a poor prognosis in HL. Numerous tryptase positive mast cells were present in reactive infiltration of the affected lymph nodes. They all expressed the CD30-L ligand, whichat confirms their involvement in HR-S regulation. The same authors proved in vitro stimulation of H-RS cells by tryptase positive mast cells and the correlation of increased mast cell density with NS type and worse DFS [20].

In our study we analyzed tryptase positive mast cells as well as chymase and tryptase positive ones. The role of the later subpopulation in HL regulation has not yet been established. In many studies both subpopulations are treated as one group of tryptase positive mast cells [18]. In the analyzed material tryptase positive mast cells outnumbered chymase positive mast cells in all subtypes. Both subpopulations were present in largest numbers in NS type (MCD-T = 10.2, MCD-C = 5.6) while in LP, MC and LR the number of mast cells decreased significantly. The lowest mast cell density was noted in LD type: MCD-T = 4.1, MCD-C = 2.3. A statistically significant difference in MCD-T and MCD-C was found between NS and LD types. No statistically significant correlation was found between MCD and age, sex or stage.

In survival analysis we have not found statistically sig-

nificant relationships between survival and mast cell density. The trend to statistical significance between DFS and MCD ($p = 0,06$) might suggest a potential usefulness of increased mast cell density as a negative prognostic factor in HL, but this fact should be considered with care. In comparison to previously cited studies by Molin et. al. [9,20] our results were not so evident. A possible reason for divergence seems to be related with the difference of samples (123 and 72 cases). Larger prospective studies are definitely recommended.

Observed increased MCD in NS type together with worse OS suggests that increased mast cell activity in NS can conduct to worse prognosis (estimated probability of 10 year survival was 40% for NS and 60% for MC). Results of our study should be considered as an introduction to further research. A large retrospective study is planned to verify our initial results. Confirming retrospective observations in RCT could result in modification of current standards of the HL management.

Tryptase- and chymase- positive mast cell density in analyzed group correlates with histological type. Increased tryptase positive mast cell density in NS type might affect worse prognosis.

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