Morphometric Study of Trephine Biopsy Specimens in Aggressive and Indolent Non-Hodgkin's Lymphomas

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Morphometric study of the erythroid stem was performed in aggressive and indolent non-Hodgkin's lymphomas *vs*. other cells and tissues of the bone marrow (including the tumor tissue) before chemotherapy. Hypoplasia and abnormal maturation of the erythroid stem were particularly pronounced in diffuse infiltration of the bone marrow, which did not depend on lymphoma aggressiveness. Hypoplasia of the erythroid stem was often observed during focal infiltration of the bone marrow with lymphoma cells (despite a smaller area of tumor tissue in aggressive lymphomas than in indolent lymphomas). A decrease in the relative area of adipose tissue, smooth resorption of bone tissue, and myelofibrosis are the major changes in the bone marrow microenvironment.

Key Words: *non-Hodgkin's lymphomas; bone marrow; trephine biopsy; erythron; myelofibrosis*

Abnormalities in the erythron system and anemia observed in 60-80% patients with non-Hodgkin's lymphomas are associated with the reduced sensitivity of tumor tissue to chemotherapy and radiotherapy, damage to the internal organs, and poor prognosis of the disease [12]. Methods for the correction of erythropoiesis in lymphoproliferative diseases should be optimized to increase the efficacy of antitumor therapy and to reduce the incidence of complications. Studying the erythron system is an urgent problem. Among a variety of mechanisms for the inhibition of erythropoiesis, a direct cytotoxic effect of tumor cells on erythron cells and indirect action of proinflammatory cytokines are of particular significance [6]. Moreover, the bone marrow microenvironment plays an important role in the regulation of erythropoiesis [1].

A morphometric study was performed to compare the erythroid stem in non-Hodgkin's lymphomas with other compartments of the bone marrow at various degrees of tumor infiltration.

MATERIALS AND METHODS

Trephine biopsy specimens were obtained from 53 patients with aggressive and indolent non-Hodgkin's lymphomas and tumor damage to the bone marrow. We examined 31 men and 22 women (26-76 years, mean age 55.07±1.96 years) that were admitted to the Novosibirsk State Regional Clinical Hospital in 2007-2010. The diagnosis of lymphoma was made according to the WHO classification (2001). A group of aggressive lymphomas (19 patients) was presented by diffuse large B-cell, mantle cell, peripheral T-cell, and Burkitt's lymphomas. A group of indolent lymphomas (34 patients) included small lymphocytic non-Hodgkin's lymphoma, lymphoplasmacytic lymphoma, and extranodal MALT lymphoma. The patients with lymphomas were examined before chemotherapy. The control group consisted of 10 patients (6 men and 4 women at the age of 38-65 years, mean age 53.5±2.61 years). The diagnosis of hemoblasto-

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sis in these patients was excluded during complex examination.

Trephine biopsy specimens from the iliac bone were fixed in 4% paraformaldehyde. Paraffin sections (4 μ) were stained with hematoxylin and eosin and examined under an Axioscop 40 microscope (Carl Zeiss). Microphotographs were made using a Canon digital camera and AxioVision Rel. 4.7.1 software. The relative areas of hemopoietic, adipose, and bone tissues, sinusoids, and tumor tissue were measured in 20 random fields of view at ×400. The type of tumor damage to the bone marrow (lymphoma cell infiltration) was estimated [7].

Nucleated cells were counted for quantitative study of the erythron. The mean diameter of at least 100 cells from each population was measured. The numerical density of cells was calculated as follows [5]: $N_V=N_A/(D+t)$, where N_A is the number of nuclear profiles in the test area, D is nucleus diameter, and t is section width. Cell number in the test area was translated into the cell number per 1 mm^3. The severity of myelofibrosis was evaluated according to the European consensus scale [15]. These sections were stained after van Gieson and impregnated with silver after Gomory. The results were analyzed using SPSS 17.0 software.

RESULTS

The relative area of bone marrow cells (normal and tumor cells) was significantly increased in aggressive (55.86±9.00%) and indolent lymphomas (55.67±5.40%) as compared to the control group (36.30±2.56%, $p<0.05$). The relative area of adipose tissue (aggressive lymphomas, 26.29±9.07%; indolent lymphomas, 25.90±5.42%) and bone tissue (aggressive lymphomas, 12.43±2.13%; indolent lymphomas, 12.33±0.95%) in lymphoma patients of both groups was lower than in control patients (38.50±3.14 and 20.0±2.60%, respectively). The bone tissue was characterized by smooth resorption in 75% patients (Fig. 1, *a*).

The relative area of sinusoids in patients with aggressive and indolent lymphoma (5.43±0.61 and 5.90±0.36%, respectively) practically did not differ from the control (5.20±0.97%). Myelofibrosis of different severity was observed in 50% trephine biopsy specimens. Grade I myelofibrosis was most incident. No differences were found in the incidence of grade II and III myelofibrosis (Fig. 1, *b*).

Morphometry of the tumor tissue showed that localized tumors of the bone marrow are present in more than 50% patients with aggressive and indolent lymphomas (numerous intertrabecular or paratrabecular clusters of tumor cells; Fig. 2, *a*). The relative area of tumor tissue during localized lymphomas of the bone marrow in patients with indolent non-Hodgkin's lymphomas (31.38±5.34%) was 2.5-fold greater than in patients with aggressive lymphomas (13.50±2.16%, $p=0.032$).

Diffuse involvement of the bone marrow was identified in 26.4% patients with lymphomas. Massive infiltration with lymphoma cells was revealed in more than 50% intertrabecular space. The area of adipose and hemopoietic tissues was significantly reduced (Fig. 2, *b*). Interstitial involvement of the bone marrow was typical of 13.2% patients. Lymphoma cells were localized between normal bone marrow cells. Normal cytoarchitectonics of the bone marrow remained unchanged under these conditions.

Quantitative study of cell populations in the bone marrow showed that the total number of bone marrow cells in patients with aggressive and indolent lymphomas is much higher than in the control ($p<0.05$, Table 1). It should be emphasized that the count of

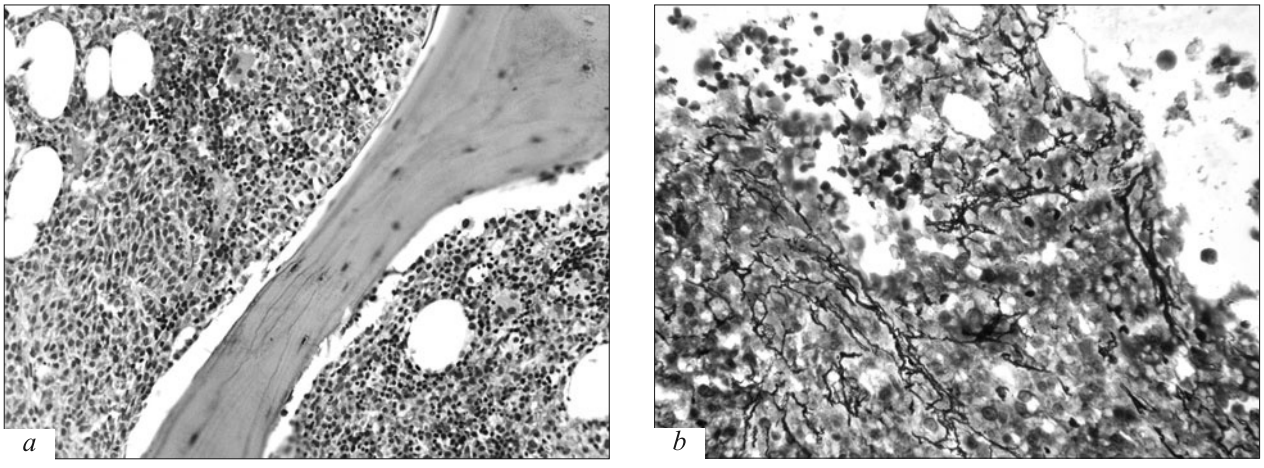

Fig. 1. Large B-cell non-Hodgkin's lymphoma. Trephine biopsy specimens from the iliac bone. (*a*) Smooth resorption of the bone trabecula. Hematoxylin-eosin staining (×400). (*b*) Grade I myelofibrosis. Silver impregnation after Gomory (×400).

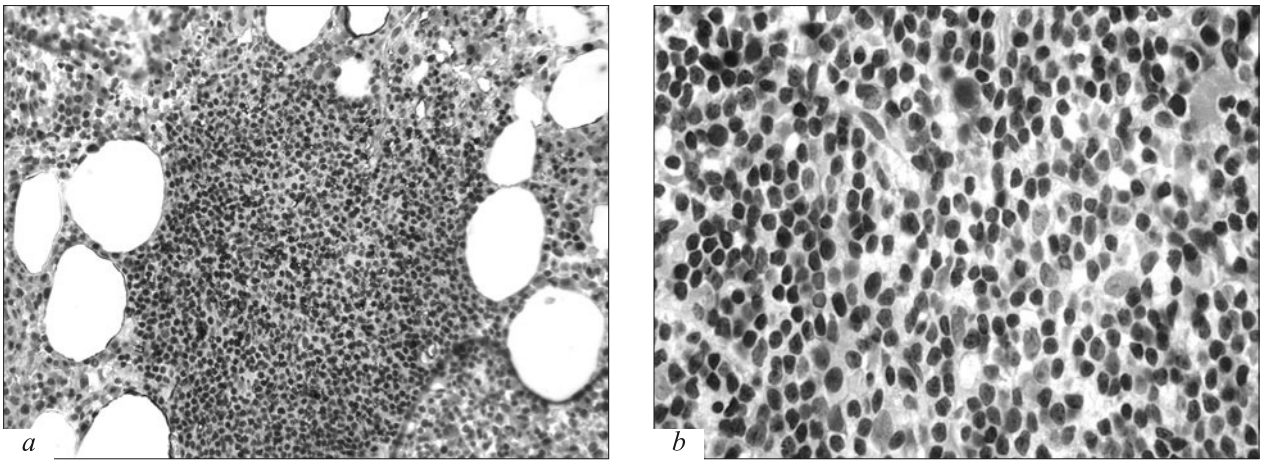

Fig. 2. Small cell non-Hodgkin's lymphoma. Trephine biopsy specimens from the iliac bone. Hematoxylin-eosin staining. Focal type of tumor cell infiltration in the bone marrow (×400, *a*). Diffuse type of tumor cell infiltration in the bone marrow (×630, *b*).

granulocytes and erythroid cells in patients with non-Hodgkin's lymphomas did not depend on tumor aggressiveness and was much lower than in the control ($p<0.05$).

During aggressive lymphomas, hypoplasia of the erythron was characterized by a decrease in the number of all cells. Indolent lymphomas were accompanied by an increase in the number of normal basophilic cells and decrease in the count of polychromatophilic and oxyphilic cells ($p<0.05$ compared to the control). The number of megaloblasts increased more significantly in aggressive lymphomas than in indolent lymphomas ($p=0.011$). Independently on the aggressiveness of non-Hodgkin's lymphoma, the number of normal lymphoid cells exceeded the control level ($p<0.05$). The count of atypical lymphocytes in indolent lymphomas was higher than that in aggressive lymphomas ($p=0.005$).

Evaluation of erythron characteristics in different types of bone marrow involvement showed that during diffuse infiltration of the bone marrow with lymphoma cells, the reduction of erythroid stem was associated with hypoplasia of the granulocyte and megakaryocyte stems (Fig. 3). Focal infiltration of the bone marrow was accompanied by polymorphism of the erythron, which depended on lymphoma aggressiveness.

The patients with indolent lymphomas were characterized by hypoplasia of the erythroid stem (as compared to the control group). Hyperplasia of various cell populations of the erythron or hyperplasia and abnormal maturation of the erythron (increase in the relative number of erythroblasts and normal basophilic cells) was rarely observed in these patients. In some patients the number of erythroid cells did not differ from the control.

Aggressive lymphomas with diffuse involvement of the bone marrow were characterized by hypoplasia of the erythroid stem (despite a smaller area of tumor tissue as compared to that in indolent lymphomas with focal infiltration of the bone marrow). Erythron reduction in aggressive and indolent lymphomas was often accompanied by hypoplasia of the granulocyte and megakaryocyte compartments.

The interstitial type of bone marrow involvement during aggressive and indolent lymphomas was accompanied by changes in the erythron and granulocyte and megakaryocyte compartments.

Our results indicate that aggressive and indolent non-Hodgkin's lymphomas are mainly associated with focal infiltration of the bone marrow with tumor cells (60.4%). The diffuse and interstitial types of tumor cell infiltration are less typical of these patients (26.4 and 13.2%, respectively) [9,10]. The relative area of tumor tissue in focal involvement of the bone marrow is higher in indolent lymphomas than in aggressive lymphomas. High incidence of bone marrow involvement during indolent B-cell lymphomas is probably associated with a greater capacity of tumor cells for invasion into the bone marrow. It is related to the ex-

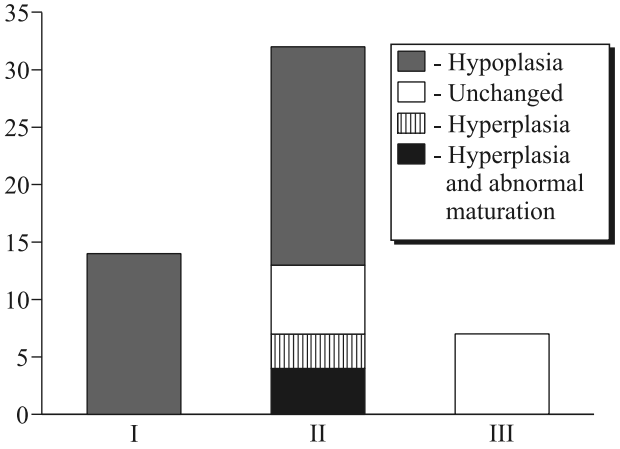

Fig. 3. State of the erythron during non-Hodgkin's lymphomas in diffuse (I), focal (II), and interstitial (III) bone marrow involvement.

TABLE 1. Number of Myelokaryocytes during Aggressive and Indolent Non-Hodgkin's Lymphomas with Bone Marrow Involvement ($M \pm m$)

Parameter	Control group (n=10)	Before therapy		p
		aggressive lymphomas (n=10)	indolent lymphomas (n=10)	
Total cellularity of the bone marrow, $\times 10^3$ cells/mm^3	90.0±7.0	105.99±10.12*	107.10±8.60*	0.795
Number of granulocytic cells, $\times 10^3$ cells/mm^3	60.03±8.54	45.52±6.15*	44.01±7.31*	0.623
Total number of erythroid cells, $\times 10^3$ cells/mm^3	18.36±2.01	15.01±1.24*	14.18±1.50*	0.194
Erythroblasts, $\times 10^3$ cells/mm^3	0.90±0.51	1.02±0.06	1.07±0.05	0.058
Normal basophilic cells, $\times 10^3$ cells/mm^3	3.61±1.03	1.80±0.13*	5.36±1.00*	<0.001
Normal polychromatophilic cells, $\times 10^3$ cells/mm^3	12.32±1.36	9.54±0.99*	6.08±1.01*	<0.001
Normal oxyphilic cells, $\times 10^3$ cells/mm^3	0.63±0.02	0.53±0.04*	0.50±0.01*	0.034
Megaloblasts, $\times 10^3$ cells/mm^3	0.90±0.10	2.12±1.06*	1.17±0.02*	0.011
Number of normal lymphoid cells, $\times 10^3$ cells/mm^3	10.71±1.56	17.26±4.32*	18.21±3.37*	0.590
Number of atypical lymphocytes, $\times 10^3$ cells/mm^3	-	22.38±5.06	29.63±5.0	0.005
Number of megakaryocytes per 1 mm^3	60.0±9.85	45.0±4.32*	42.0±6.04*	0.205

Note. *p<0.05 compared to the control group.

pression of adhesion molecules that provide cell tropism for hemopoietic tissue and connective tissue cells of the bone marrow [7,14].

Studying the effect of tumor tissue on erythropoiesis during aggressive and indolent non-Hodgkin's lymphomas allowed describing the "syndrome of occupied place" during leukemia cell invasion into the bone marrow [3]. Hypoplasia of normal hemopoietic stems and decrease in the area of adipose tissue are typical of diffuse involvement of the bone marrow with the greatest area of tumor tissue. However, the inhibition of erythropoiesis cannot be associated only with the expansion of tumor infiltrate.

Recent studies showed that reduced production of erythropoietin, action of proinflammatory cytokines that induce abnormal erythropoiesis, and disturbances in iron metabolism serve as the mechanisms, which have an adverse effect on the bone marrow erythron [8,13]. These factors probably have a greater role during aggressive lymphomas compared to indolent tumors.

Our previous studies revealed that aggressive and indolent non-Hodgkin's lymphomas are characterized by different correlations between the degree of tumor lesion in the bone marrow and quantitative parameters of the erythron [2]. A direct correlation was found between an increase in the number of tumor cells and hypoplasia of the erythron during indolent lymphomas. By contrast, this correlation was not observed during aggressive lymphomas. For example, the incidence of hypoplasia of the erythroid stem during focal infiltration of the bone marrow with lymphoma cells was similar in patients with aggressive and indolent lymphomas (despite a smaller area of tumor tissue in aggressive lymphomas than in indolent lymphomas).

The development of myelofibrosis in 50% trephine biopsy specimens from patients with non-Hodgkin's lymphomas plays an important role in cell-cell interaction for regulation of hemopoiesis [4,7,11].

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