Morphological Characteristics of the Central Compartment of the Erythron in Aggressive and Indolent Non-Hodgkin's Lymphomas

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Structural changes in cells of the central compartment of the erythron depended on tumor infiltration of the bone marrow. Cytostatic therapy was shown to improve quantitative indexes, but had no effect on morphological characteristics of the erythron. Examination of trepanobiopsy specimens from the iliac crest during chemotherapy revealed the existence of specific relationships between the erythron, other cells and tissues of the bone marrow, and tumor. Significant changes in the erythron were found during diffuse tumor infiltration and treatment with anthracycline antibiotics.

Key Words: *erythron; bone marrow; aggressive lymphomas; indolent lymphomas; trepanobiopsy*

Anemia develops in 60-80% patients with non-Hodgkin's lymphomas. This disorder serves as an unfavorable prognostic criterion, which reduces the efficiency of chemotherapy and radiation therapy [6,8-10]. Much attention was paid to studying of the erythron in various lymphoproliferative diseases [1,3]. However, little is known about the dependence of morphological changes in erythroid cells on tumor infiltration of the bone marrow. The interaction of the erythroid stem with other cells and tissues of the bone marrow during chemotherapy remains unclear [4,5,7].

Due to the high prognostic significance of anemia in lymphoproliferative diseases, we compared the quantitative and structural changes in the central compartment of the erythron and studied the effect of various types of tumor infiltration in the bone marrow on the erythroid stem in patients with aggressive and indolent non-Hodgkin's lymphomas before and during chemotherapy.

MATERIALS AND METHODS

We examined 120 patients (16-80 years) with aggressive non-Hodgkin's lymphomas (61 men and 59 women, mean age 50.33±1.54 years) and 93 patients (20-78 years) with indolent non-Hodgkin's lymphomas (54 men and 39 women, mean age 57.72±1.54 years). All patients were admitted at the Novosibirsk regional clinical hospital in 2000-2010. The diagnosis of lymphoma was made on the basis of light microscopy and immunohistochemical examination of biopsy specimens from the lymph nodes or other organs (extranodal lymphoma). Specific therapeutic schemes were applied to patients with aggressive lymphomas (CHOP, CHOEP, RCHOP) and indolent lymphomas (COP, RCOP, CVP, RCVP, and LVP). Complete or partial remission was achieved after 4-6 courses of chemotherapy.

Cytological analysis of bone marrow aspirate was performed (Romanovsky–Giemsa staining). The fol-

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lowing abnormalities of bone marrow erythrokaryocytes were used as morphological criteria of impaired erythropoiesis: presence of megaloblastoid, binucleated, and multinucleated cells, dissociation of maturation of the nucleus and cytoplasm, intercellular bridges, Jolly's bodies, Cabot's rings, fragmentation of the nuclei, and basophilic punctuation. Quantitative indexes of the central hemopoiesis compartment were evaluated (percentage of erythroid cells, erythroblasts, basophilic, polychromatophilic, and oxyphilic normocytes, and megaloblasts). Paraffin sections of trepanobiopsy specimens from the iliac crest were stained with hematoxylin and eosin (or by the method of van Gieson). We estimated the relative area of hemopoietic, adipose, and bone tissue and sinusoidal vessels, maturation of the erythroid, granulocytic, and megakaryocytic stem, type of tumor infiltration in the bone marrow, and relative area of the tumor. The study was conducted under an Axioscop 40 laboratory microscope (Carl Zeiss). Microphotographs were obtained using a Canon digital camera and AxioVision Rel. 4.7.1 software. The results were analyzed by SPSS 17.0 software.

RESULTS

Comparative study of the central compartment of the erythron before cytostatic treatment (Table 1) revealed a significant decrease in all cell populations of the erythron in 73 and 54% patients with aggressive and indolent lymphomas, respectively ($p<0.05$). The patients with aggressive lymphomas were characterized by a decrease in the number of erythroblasts (36% patients), basophilic normocytes (69% patients), and polychromatophilic (59% patients) and oxyphilic normocytes (21% patients). Abnormal erythropoiesis manifested in dissociation of maturation of the nucleus and cytoplasm (42% patients) and the presence of intercellular bridges (24% patients), Jolly's bodies (32% patients), and multinucleated erythrokaryocytes (18%

TABLE 1. Indexes of the Erythron in Non-Hodgkin's Lymphomas with Bone Marrow Infiltration during Chemotherapy (*M*±*m*)

Erythron indexes		Control group (*N*=10)	Before therapy		During therapy	
			patients with aggressive lymphomas (*N*=39)	patients with indolent lymphomas (*N*=47)	patients with aggressive lymphomas (*N*=20)	patients with indolent lymphomas (*N*=23)
Erythroblasts, %		0.66±0.14	0.37±0.09^{+}	0.27±0.30^{+}	0.44±0.70*	0.56±0.07*
Normocytes, %	basophilic	2.88±0.47	1.05±1.13^{+}	2.0±0.57^{+}	0.91±0.22^{+}	3.28±1.00*
	polychromatophilic	11.58±0.77	8.06±0.72^{+}	8.13±0.80^{+}	9.98±1.98^{+*}	12.16±0.96*
	oxyphilic	4.64±0.46	2.58±0.41^{+}	2.45±0.23^{+}	2.53±0.64^{+}	2.92±0.43^{+}
Megaloblasts, %		1.70±0.37	1.18±0.25	0.98±0.23	1.16±2.26	1.16±0.26

Note. Here and in Table 2: $p<0.05$: *compared to the corresponding index before chemotherapy; $^{+}$compared to the control group.

TABLE 2. Indexes of the Erythron in Non-Hodgkin's Lymphomas without Bone Marrow Infiltration during Chemotherapy (*M*±*m*)

Erythron indexes		Control group (*N*=10)	Before therapy		During therapy	
			patients with aggressive lymphomas (*N*=49)	patients with indolent lymphomas (*N*=29)	patients with aggressive lymphomas (*N*=29)	patients with indolent lymphomas (*N*=18)
Erythroblasts, %		0.66±0.14	0.45±0.08^{+}	0.54±0.30^{+}	0.50±0.72*	0.59±0.07*
Normocytes, %	basophilic	2.88±0.47	1.65±1.13^{+}	2.03±0.57	1.98±0.24*	3.29±1.04*
	polychromatophilic	11.58±0.77	8.96±0.74^{+}	8.15±0.80^{+}	9.98±1.95*	11.16±0.97*
	oxyphilic	4.64±0.46	2.96±0.42^{+}	3.01±0.27^{+}	3.33±0.66*	3.93±0.44*
Megaloblasts, %		1.70±0.37	1.20±0.25	1.00±0.25	1.72±1.26*	1.25±0.25

patients). The patients with indolent lymphomas were characterized by similar quantitative and qualitative changes in the central compartment of the erythron.

Chemotherapy (4-6 courses) for aggressive and indolent lymphomas with bone marrow infiltration was followed by a significant decrease in the incidence of erythropoiesis suppression due to an increase in the number of polychromatophilic normocytes ($p<0.05$).

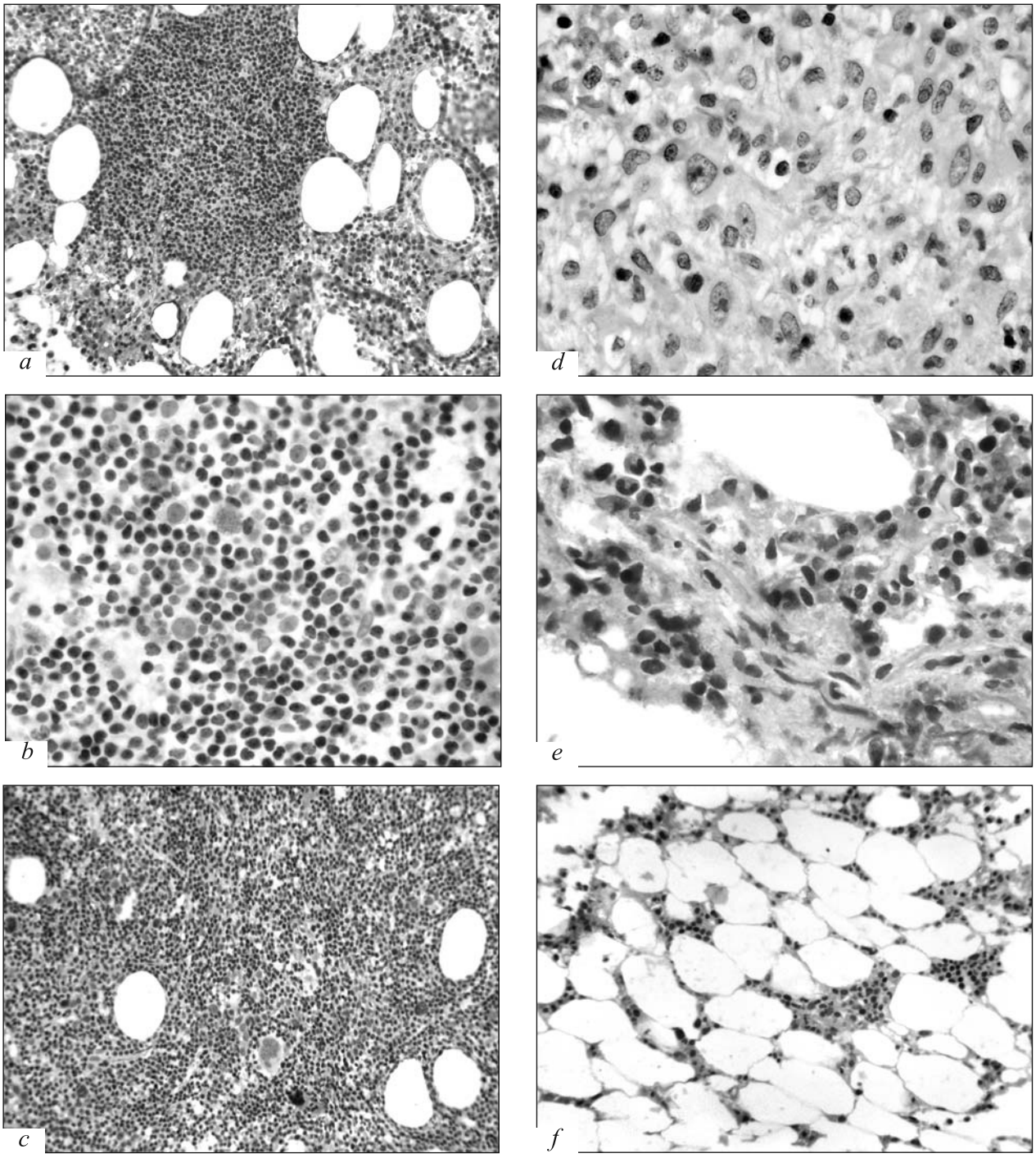

Fig. 1. Structural changes in the central compartment of the erythron in aggressive and indolent non-Hodgkin's lymphomas. Trepanobiopsy specimens from the ileum. Hematoxylin-eosin staining, ×200 (*a*, *c*, *f*), ×630 (*b*, *d*, *e*). *a*: Follicular non-Hodgkin's lymphoma. Local infiltration of the bone marrow with tumor cells. *b*, *c*: Non-Hodgkin's lymphoma of small lymphocytes. Diffuse infiltration of the bone marrow with small lymphocyte-like cells. *d*: Giant cell anaplastic lymphoma. Diffuse infiltration of the bone marrow with large tumor cells. *e*: Non-Hodgkin's lymphoma of small lymphocytes. Diffuse and infiltrative infiltration of the bone marrow with small lymphocyte-like cells. *f*: Burkitt's non-Hodgkin's lymphoma. Total substitution of bone marrow cells for adipose tissue.

The count of basophilic normocytes returned to normal in the majority of patients with indolent lymphomas ($p<0.05$). Several signs of abnormal erythropoiesis (*e.g.*, Jolly's bodies, intercellular bridges, dissociation of maturation of the nucleus and cytoplasm, and multinucleated erythrokaryocytes) were shown to persist in patients with aggressive and indolent lymphomas. Pathomorphological changes in the bone marrow compartment of the erythron were similar in patients with aggressive and indolent lymphomas. They did not depend on the presence or absence of tumor dissemination in the bone marrow (Table 2). It should be emphasized that tumor infiltration of the bone marrow in patients of both groups serves as the factor associated with reduction of the bone marrow erythroid stem ($p<0.05$). Apart from the persistence of abnormal erythropoiesis in aggressive lymphomas (irrespective of tumor dissemination in the bone marrow), cytostatic treatment was followed by an increase in the number of megaloblasts in myelograms (from 4 to 16%; $p<0.05$).

Studying the relationships of the erythron with other cells and tissues of the bone marrow was performed by morphological examination of biopsy specimens from the iliac crest. In lymphoma patients without bone marrow infiltration, the relative area of bone marrow cells before chemotherapy was normal (85% patients) or reduced (15% patients). A positive correlation between the relative areas of bone marrow cells, erythroid stem, granulocytic stem, and megakaryocytic stem was observed in most patients. The decrease in the relative area of bone marrow cells was accompanied by erythroid hypercellularity (stimulation of the erythroid stem) in 2% patients. Maturation of erythroid cells into normoblast was found in 80% patients. Rejuvenation of the erythroid stem (*i.e.*, increase in the relative number of erythroblasts and pronormocytes) was typical of 20% patients.

The development of non-Hodgkin's lymphomas with metastatic involvement of the bone marrow was accompanied by the following types of bone marrow lesion: local (75%; Fig. 1, *a*), diffuse (10%; Fig. 1, *b-d*), complex (10%; Fig. 1, *e*), and interstitial injury (5%). The most pronounced changes in the erythron were observed in diffuse infiltration. Hypoplasia of the erythroid stem (100%) was associated with displacement of the granulocytic and megakaryocytic lineage cells and decrease in the relative area of adipose tissue. The major changes during local injury manifested in hypoplasia of all hemopoietic lineages (76% patients). Rejuvenation and stimulation of the erythroid stem were found in 10% patients. The central compartment of the erythron remained unchanged in 14% patients. The relative area of bone marrow cells under conditions of local infiltration was normal or increased in various compartments of the bone marrow, which depended on the relative area of tumor tissue. No changes in the erythron were identified during interstitial lesion to the bone marrow. The severity of changes in the erythron during complex infiltration with tumor cells was shown to vary significantly and depended on the major type of lymphoid injury.

Hemopoiesis returned to normal in 90% patients without bone marrow infiltration with partial or complete remission after 4-6 courses of chemotherapy. The decrease in the relative area of bone marrow cells in 5% patients was related to the inhibition of cell populations and increase in the relative area of adipose tissue. The recovery of hemopoiesis was observed in 70% patients with diffuse tumor infiltration. Hypoplasia of the erythron and/or granulocytic and megakaryocytic stem was found in 22% patients. The bone marrow was substituted for adipose tissue in 8% patients of this group (Fig. 1, *f*). The relative area of bone marrow cells returned to normal in patients with local tumor infiltration of the bone marrow at the stage of remission. Many patients were characterized by hyperplasia (stimulation) and dysplasia of the erythroid and megakaryocytic stems, respectively.

Our results indicate that changes in the central compartment of the erythron do not depend on aggressiveness of lymphoma. Chemotherapy is not accompanied by significant changes in the bone tissue or relative area of sinusoidal vessels. Activity of the erythron is mainly determined by the presence and type of tumor infiltration in the bone marrow. The most pronounced changes are observed during diffuse infiltration due to displacement of normal cells by tumor cells. The development of non-Hodgkin's lymphomas is accompanied by a shift in the cytokine ratio. These patients have the increased concentration of IFN-γ [2], which produces an inhibitory effect on erythropoiesis. The cytokine imbalance probably modulates the intensity of erythropoiesis, which is followed by hyperplasia, stimulation, and/or rejuvenation of the erythron. The inhibition of hemopoietic stems in non-Hodgkin's lymphomas without bone marrow infiltration is probably related to cytokine production by extramedullary tumor cells.

Cytostatic treatment was accompanied by complete recovery of erythroid cells (particularly in local lesion to the bone marrow) or total substitution of bone marrow cells for adipose tissue (lymphomas with diffuse metastatic dissemination). Moreover, chemotherapy was followed by reactive hyperplasia of the erythron and prevalence of young cells. These changes were often related to the treatment with anthracycline antibiotics. These data suggest that activity of the erythron depends not only on the type of tumor infiltration in the bone marrow, but also on the scheme of chemotherapy.

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