Erythropoiesis in Patients with Aggressive and Indolent Non-Hodgkin's Lymphomas

N. P. Domnikova, V. V. Degtyareva, and L. M. Nepomnyashchikh

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 12, pp. 668-673, December, 2004
Original article submitted July 28, 2004

Parameters of the erythroid, granulocytic, and megakaryocytic hemopoietic stems were compared in 87 patients with aggressive and indolent non-Hodgkin's lymphomas before and 6 months after the start cytostatic therapy. Before chemotherapy anemia was detected in 46% patients with aggressive and 49% patients with indolent lymphomas. Hemoglobin content, peripheral blood erythrocyte count, and total count of erythroid cells in the bone marrow increased during chemotherapy in the indolent lymphoma group. Increased count of erythroid cells in the myelogram was due to decreased count of lymphoid cells in the bone marrow, which was associated with complete or partial remission. In aggressive lymphoma chemotherapy decreased the mean level of hemoglobin and mean erythrocyte count in the peripheral blood, but the total count of erythroid cells in the bone marrow increased; no relationship was detected between lymphocyte count in the bone marrow and erythropoiesis characteristics. Lymphocytosis >50% in the myelogram before chemotherapy was less frequent in this group in comparison with indolent non-Hodgkin's lymphomas.

Key Words: *non-Hodgkin's lymphomas; hemopoiesis; anemia; chemotherapy; hemograms; myelograms*

Anemia in patients with malignant tumors remains a pressing problem of oncology and hematology. This frequent complication of lymphoproliferative diseases deteriorates the tolerance of antitumor treatment, impairs quality of life, and reduces life span [8-10,14].

The characteristics of the hemopoietic erythroid stem in patients with indolent and aggressive non-Hodgkin's lymphomas (NHL) were never compared. The data on the pathogenetic mechanisms of the anemic syndrome in indolent and aggressive NHL are scanty. The mechanism of development or progress of anemia in NHL patients during cytostatic therapy is also poorly understood [11,13,15].

Laboratory of Molecular Cellular and Immunomorphological Bases of Oncohematology, Institute of Regional Pathology and Pathomorphology, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** pathol@soramn.ru. N. P. Domnikova

We studied erythropoiesis in patients with aggressive and indolent NHL before and during cytostatic therapy.

MATERIALS AND METHODS

A total of 87 patients with NHL (53 males and 31 females ones, mean age 56.7 years) were examined. The patients were divided into groups with aggressive (n=46) and indolent (n=41) NHL. All patients were examined and treated at State Novosibirsk Regional Clinical Hospital.

The disease was diagnosed in all cases in accordance with the working formulation (1982); stages of NHL were diagnosed in accordance with Ann Arbor classification (1971). Bone marrow involvement was detected in 12 patients with aggressive NHL and 32 with indolent NHL.

Patients with aggressive NHL received combined chemotherapeuty according CHOP, CHOEP, BACOD,

0007-4888/04/1386-0593

or RACOP protocols. Patients with indolent NHL received cyclophosphamide monotherapy, COP and CHOP protocols. Chemotherapy efficiency was evaluated by common criteria 6 months after diagnosis and start of therapy [1]. Cytostatic therapy led to complete and partial remission in 24 cases in both groups.

Peripheral blood smears and bone marrow specimens stained by the method of Romanowskii—Giemsa were prepared before and after 6-month chemotherapy. Parameters reflecting normal hemopoiesis status were correlated to lymphocyte count in the bone marrow. For studies of the hemopoietic erythroid, granulocytic, and megakaryocytic stems, the patients were divided into groups basing on the normal values of the hemogram and myelogram [2].

The results were processed using Epi Info software. Variables were compared using Student's *t* test and ANOVA; the Kruskal—Wallis nonparametrical test was used for abnormal distribution. Contingency tables were used for the analysis of the distribution frequencies; the logistic regression model for odds ratio (OR) evaluation and estimation of 95% confidence interval (CI) was used for evaluating the prognostic significance of variables. Large samples were analyzed using χ^2 test by the Mantel—Hanszel method with Yates' correction, small samples were analyzed by precise Fisher's test. The relationship between variables was evaluated by the coefficient of correlations (r). The differences were considered significant at $p<0.05$.

RESULTS

Before chemotherapy anemia was diagnosed in 41 of 87 patients (47.1%): severe in 1 (1.1%), medium in 11 (12.6%), and mild in 29 (33.3%) patients.

The incidence of the anemic syndrome was virtually the same in the two groups before chemotherapy

TABLE 1. Hemogram and Myelogram of Patients with Aggressive and Indolent NHL before Chemotherapy

Parameter		Patients with NHL		Total	Significance of differences, p
		aggressive	indolent		
Hemogram					
hemoglobin	below 120 g/liter	21	20	41	0.939
	≥120 g/liter	25	21	46	
erythrocytes	$<4.0\times10^{12}$/liter	22	23	45	0.578
	$\geq4.0\times10^{12}$/liter	24	18	42	
leukocytes	$>9.0\times10^{9}$/liter	30	15	45	0.014
	$\geq9.0\times10^{9}$/liter	16	26	42	
lymphocytes	<40%	34	16	50	0.002
	≥40%	12	25	37	
segmented neutrophils	<45%	17	26	43	0.025
	≥45%	29	15	44	
monocytes	<9%	35	36	71	0.258
	≥9%	11	5	16	
platelets	$<150.0\times10^{9}$/liter	13	10	23	0.869
	$\geq150.0\times10^{9}$/liter	33	31	64	
Myelogram					
erythroid cells	<14.5%	24	28	52	0.190
	≥15.5%	22	13	35	
lymphocytes	<14%	27	16	43	0.106
	≥14%	19	25	44	
monocytes	<3%	39	35	74	0.822
	≥3%	7	6	13	
myelocytes	<7%	13	26	39	0.002
	≥7%	33	15	48	
stab neutrophils	<12.8%	35	33	68	0.813
	≥12.8%	11	8	19	
segmented neutrophils	<13%	14	19	33	0.192
	≥13%	32	22	54	

TABLE 2. Hemogram and Myelogram before and after Chemotherapy in Patients with Aggressive and Indolent NHL (*M*±*m*)

Parameter		Patients with NHL		
		aggressive	indolent	total
Hemogram				
hemoglobin, g/liter	before chemotherapy	120.22±3.68	120.63±3.40	120.41±2.47
	after chemotherapy	116.37±3.81*	126.08±3.36*	120.99±2.50
erythrocytes, $\times 10^{12}$/liter	before chemotherapy	3.89±0.11	3.84±0.11	3.87±0.08
	after chemotherapy	3.79±0.13*	4.03±0.10*	3.90±0.76*
Myelogram				
erythroid cells, %	before chemotherapy	15.32±1.33	11.54±1.72	13.54±0.9
	after chemotherapy	16.27±1.87**	16.1±1.6*	16.14±1.20*
lymphocytes, %	before chemotherapy	21.30±3.54	41.46±6.40	30.8±3.1
	after chemotherapy	22.71±3.90	31.47±4.10*	26.98±2.90*

Note. *p<0.001, **p<0.01 compared to the corresponding parameters before chemotherapy.

(Table 1): 21 (46%) patients in the aggressive and 20 (49%) in the indolent NHL groups. The number of patients with low hemoglobin level, erythrocyte counts in the peripheral blood, and decreased counts of erythroid cells in the bone marrow was the same in the two groups (Table 1); mean hemoglobin levels and erythrocyte counts in the peripheral blood were the same (Table 2). On the other hand, myelograms of patients with aggressive NHL showed higher counts of erythroid cells (15.32±1.47% in aggressive and 11.54± 1.41% cells in indolent NHL, p<0.001).

Chemotherapy decreased the mean hemoglobin level in aggressive NHL and increased it in indolent NHL. Anemia (hemoglobin <120 g/liter) was more often observed before therapy than after it (47 and 40%, respectively, p<0.001). These changes were most pronounced in indolent NHL (p<0.05; Fig. 1). The

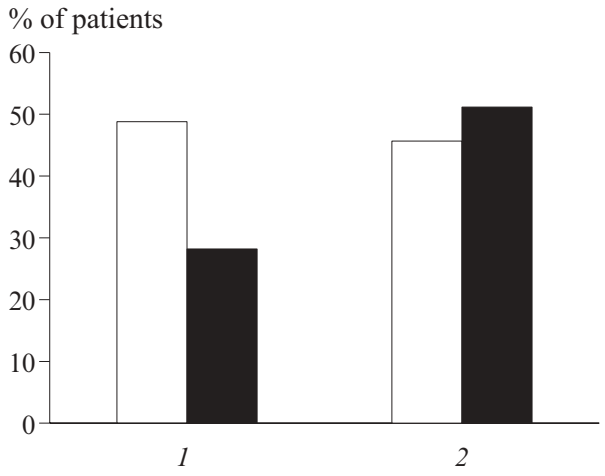

Fig. 1. Incidence of anemic syndrome in patients with aggressive (dark bars) and indolent (light bars) non-Hodgkin's lymphomas before (*1*) and after (*2*) chemotherapy.

incidence of the anemic syndrome in the patients with aggressive NHL before and after chemotherapy was virtually the same.

Cytostatic therapy increased the counts of erythroid cells in myelograms of all patients and decreased lymphocyte counts in the bone marrow (Table 2). Similar changes in hemoglobin level, erythrocyte count, and erythroid cells were recorded in myelograms of patients with indolent NHL, in contrast to patients with aggressive NHL in whom these shifts were oppositely directed.

Hemoglobin level and erythrocyte counts in the peripheral blood of patients with aggressive NHL decreased after chemotherapy, while the total count of erythroid cells in the bone marrow increased. In patients with indolent NHL chemotherapy increased (*vs.* the initial levels) parameters characterizing erythropoiesis (mean hemoglobin level, erythrocyte count in the peripheral blood, and total count of erythroid cells in the bone marrow).

The initial values of hemogram and myelogram reflecting the status of other hemopoietic stems were different in the two groups before cytostatic therapy (Table 1). Indolent NHL were more often associated with changes caused by tumor infiltration of the bone marrow: leukocytosis with lymphocytosis and granulocytopenia in differential leukocyte acount, decreased myelocyte count in the myelogram. An inverse relationship between lymphocyte and erythroid cell counts in myelogram was detected (r=-0.44). Lymphocyte content >50% in the bone marrow of patients with indolent NHL was a factor associated with a decrease in the total number of erythroid cells in the myelogram below 14.5% (p=0.014).

The detected reduction of the hemopoietic erythroid stem in patients with indolent NHL with more

than 50% lymphocytes in the bone marrow prompted us to investigate the effect of this degree of lymphocytosis on other hemopoietic stems and hemogram parameters (Table 3). Bone marrow lymphocytosis >50% before chemotherapy was associated with thrombocytopenia and granulocytopenia (according to hemograms) and with decreased count of granulocytic stem cells in the myelogram (myelocytes, stab and segmented neutrophils).

Before chemotherapy patients with indolent NHL with and without >50% lymphocytosis in the myelogram did not differ by the levels of hemoglobin, erythrocyte and monocyte counts in the peripheral blood, and monocyte counts in the bone marrow. On the other hand, the increase in the counts of erythroid cells in the myelogram after chemotherapy was associated with a decrease in the count of lymphoid cells in the bone marrow (r=-0.51). Decreased count of lymphoid cells in the myelogram as a result of cytostatic therapy was associated with complete and partial remission (OR=2.43, 95% CI 1.14-5.20; p=0.012).

No relationship between lymphocyte content in the bone marrow and parameters characterizing erythropoiesis was detected in the patients with aggressive NHL. Lymphocytosis >50% in the myelogram before chemotherapy was recorded in 6 of 46 patients, which was much more rare (p=0.011) than in patients with indolent NHL.

Presumably, the development of anemia in aggressive NHL is associated with a negative effect of the tumor clone on erythropoiesis mediated by excessive secretion of proinflammatory cytokines (IL-1, IL-6, TNF-α). These cytokines inhibit the production of erythropoietin (stimulating division and maturing of erythroid precursor cells in the bone marrow), promote the development of redistributional deficit of iron (intracellular accumulation of this element in tissue macrophages in the presence of its decreased content in the blood serum [5,12]).

Anemia often develops or augments during cytostatic therapy, which can be explained by the negative effect of the absolute majority of clinically used antitumor drugs on erythropoiesis [3,4]. Anemia after chemotherapy courses was observed in 22 patients with aggressive NHL. In 9 of these 22 cases decreased hemoglobin level and erythrocyte count in the peripheral

TABLE 3. Hemogram and Myelogram and Bone Marrow Lymphocyte Count in NHL Patients before Chemotherapy

Parameter		Number of patients with lymphocyte percentage in myelogram		
		≥50% (n=16)	<50% (n=25)	OR
Hemoglobin	<90 g/liter	3	2	1.66
	≥90 g/liter	13	23	(0.72-3.84)
Erythrocytes in hemogram	$<4.0\times10^{12}$/liter	9	14	1.01
	$\geq4.0\times10^{12}$/liter	7	11	(0.24-4.31)
Platelets in hemogram	$<150.0\times10^{9}$ g/liter	8	2	3.10
	$\geq150.0\times10^{9}$ g/liter	8	23	(1.58-6.07)
Segmented neutrophils in hemogram**	<45%	16	10	—
	≥45%	0	15	
Monocytes in hemogram	<9%	15	21	2.08
	≥9%	1	4	(0.35-12.54)
Erythroid cells in myelogram	<14.5%	15	13	6.96
	≥14.5%	1	12	(1.03-47.24)
Myelocytes in myelogram	<7%	15	11	8.65
	≥7%	1	14	(1.27-59.14)
Stab neutrophils in myelogram*	<12.8%	16	17	—
	≥12.8%	0	8	
Segmented neutrophils in myelogram**	<13%	13	6	5.02
	≥13%	3	19	(1.68-15.00)
Monocytes in myelogram	<3%	15	20	2.57
	≥3%	1	5	(0.41-16.02)

Note. *p<0.05, **p<0.001. 95% CI is shown in parentheses.

blood were associated with high total count of erythroid cells in the myelogram and were not caused by blood loss. In 5 of these 9 cases the anemic syndrome was initially absent and appeared only after chemotherapy. In this group hemolysis was more often recorded in patients receiving chemotherapy (in 1 of 9 cases before and in 4 of 9 cases after chemotherapy, $p<0.01$).

Decreased levels of hemoglobin and erythrocytes in the presence of increased count of erythroid cells in the bone marrow in patients with aggressive NHL can be caused by hemolysis induced by cytostatics. Some scientists noted prooxidant effects of cytostatics, which impaired metabolism and membrane structures of the peripheral blood erythrocytes and bone marrow erythrokaryocytes by stimulating free-radical oxidation, this leading to increase of hemolysis [6,9].

Hence, tumor reduction after cytostatic therapy is not associated with suppression of erythropoiesis in patients with indolent NHL, treated by chemotherapy protocols with low myelotoxicity; hemoglobin level and erythrocyte counts in the peripheral blood increase, which is associated with the disease remission. In aggressive NHL the tumor effects caused by the production of humoral factors, are essential for the genesis of anemia. In these patients cytostatic therapy can be associated with persistence or development of anemia, including that with hemolysis elements.

REFERENCES

1. M. A. Volkova, *Clinical Oncohematology* [in Russian], Moscow (2001).
2. A. I. Vorob'ev, *Manual of Hematology* [in Russian], Moscow (1985).
3. M. L. Gershanovich, *Complications in Chemo- and Hormone Therapy of Malignant Tumors* [in Russian], Moscow (1982).
4. E. D. Gol'dberg and V. V. Novitskii, *Antitumor Antracyclin Antibiotics and Blood System* [in Russian], Tomsk (1986).
5. V. V. Dolgov, S. A. Lugovskaya, M. E. Pochtar', *et al.*, *Laboratory Diagnosis of Iron Metabolism Disorders* [in Russian], Moscow (1996).
6. I. V. Kostareva, *Erythropoiesis Damaging Effects of Antitumor Antracyclin Antibiotics* [in Russian], Tomsk (1987).
7. Yu. V. Rumyantseva, N. S. Smetanina, and A. G. Rumyantsev, *Vopr. Gematol. Onkol. Immunopatol. v Pediatr.*, **2**, No. 2, 52-62 (2003).
8. M. Cazzola, Y. Beguin, J. Kloczko, *et al.*, *Br. J. Haematol.*, **122**, Suppl. 3, 386-393 (2003).
9. D. C. Doll and R. B. Weiss, *Cancer Treat. Rep.*, **69**, 777-782 (1985).
10. C. Kasper, *Ann. Haemat.*, **80**, 319-329 (2001).
11. M. J. Kyasa, R. S. Parrish, S. A. Schichman, and C. S. Zent, *Am. J. Haemat.*, **74**, Suppl. 1, 1-8 (2003).
12. R. T. Means, *Stem Cells*, **13**, 32-37 (1995).
13. D. Sella and D. Bron, *Cancer Pract.*, **7**, Suppl. 4, 177-182 (1999).
14. M. P. Siakantaris, M. K. Angelopoulou, T. P. Vassilakopoulos, *et al.*, *Leuk. Lymphoma*, **40**, 141-147 (2000).
15. M. L. Thomas, *Med. Oncol.*, **15**, Suppl. 3, 13-18 (1998).