State of Central and Peripheral Pools of Hemopoietic Precursor Cells in Patients with Malignant Neoplasms during Chemotherapy

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We studied toxic effects of various schemes of cytostatic treatment on central and peripheral pools of hemopoietic precursor cells in patients with III-IV stages of lung cancer and breast cancer. It was found that erythro- and granulomonocytopoietic precursors are characterized by high resistance to cytostatic treatment compared to morphologically discernible bone marrow elements, which is probably a evolutionary developed property of this cell type aimed at rapid recovery of the hemopoietic tissue.

Key Words: *chemotherapy; granulomonocytopoiesis; etyrhopoiesis; hemopoietic precursors*

Chemotherapy is an obligatory component in the complex therapy of malignant neoplasms including also surgical methods, radio- and immunotherapy. In some neoplasms chemotherapy is the only way to attain recovery or prolongation of patient's lifespan. Many antitumor drugs differing by their chemical structure and mechanisms of action are now widely used [7]. However, due to their low tumor selectivity all these drugs applied as monotherapy, or as components of complex cytostatic therapy induce undesirable changes in normal organs and tissues. Hypo- and aplastic states of the hemopoiesis are typical manifestations of the toxic effect of antitumor drugs limiting their clinical use. These states are characterized by reduced bone marrow cellularity, decreased count of leukocytes (primarily due to decreased number of segmented neutrophils) and platelets in the peripheral blood [1].

There are ample data confirming the viewpoint that cytostatic-induced suppression of hemopoiesis results from damage to proliferating bone marrow cells and exhaustion of the pool of hemopoietic precursors. At the same time it is known that quantitative regulation of hemopoiesis during stress exposures (including cytostatic trauma) is realized at the level of committed and partially determined hemopoietic precursor cells [2].

The aim of the present study was to evaluate the state of central and peripheral pools of hemopoietic precursor cells in patients with malignant neoplasms during chemotherapy.

MATERIALS AND METHODS

We examined 80 patients with stages III-IV lung cancer, of them 33 patients received antitumor therapy by original CVC protocol (intravenous injections of 1.4 mg/m^2 vincristine, 600 mg/m^2 cyclophosphamide on day 1, and 300 mg/m^2 carboplatin on day 2), 47 patients received CAM protocol (intravenous injections of 750 mg/m^2 cyclophosphamide, 25 mg/m^2 adriamycin on days 1 and 8, and 20 mg/m^2 methotrexate on days 2 and 9).

The results of blood system testing in 40 patients with stages III-IV breast cancer were also

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included. These patients received antitumor polychemotherapy by CAF protocol (intravenous injection of 60 mg/m^2 adriamycin, 1000 mg/m^2 5-fluorouracil, and 2400 mg/m^2 cyclophosphamide). The treatment consisted of two 12-day cycles with a 2 week interval.

The bone marrow, peripheral and venous blood were examined.

Parameters of peripheral blood (hemoglobin, erythrocytes, leukocytes, and hemogram) and differential myelograms were evaluated using standard hematological methods [6].

Precursors of granulomonocytopoiesis (CFU-GM) and erythropoiesis (CFU-E) were cloned from nonadherent bone marrow and peripheral blood karyocytes (2.0×10^5 cell/ml) in a semisolid medium containing 50% McCoy 5A medium (ICN), 19% embryo calf serum (ICN), 280 mg/liter L-glutamine (Sigma), 50 mg/liter gentamicin (Serva), 0.4×10^{-5} M 2-mercaptoethanol (Sigma), 30% methylcellulose (Sigma), 5 μl/liter recombinant G-CSF (Neipogen, Hoffman la Roshe) for granulocyte colonies or 0.5 U/ml recombinant erythropoietin (Sigma) for erythroid colonies. The cell suspension was transferred to 35-mm plastic Petri dishes and incubated for 7 (CFU-GM) or 3 (CFU-E) days at 37°C, 5% CO_2, and 100% humidity. The colonies (cell aggregations consisting of at least 50 nuclears) were counted using a Biolam-P1 inverted microscope (×56). Morphology of individual colonies were studied on preparations stained with azur-II-eosin [4,9].

RESULTS

All three protocols used for the treatment of cancer patients always decreased the total leukocyte count (by 20.8, 25.5, and 5.1%, respectively) after the first course of chemotherapy (Fig. 1, *c*). After the second course this parameter progressively decreased and remained below the initial level. The

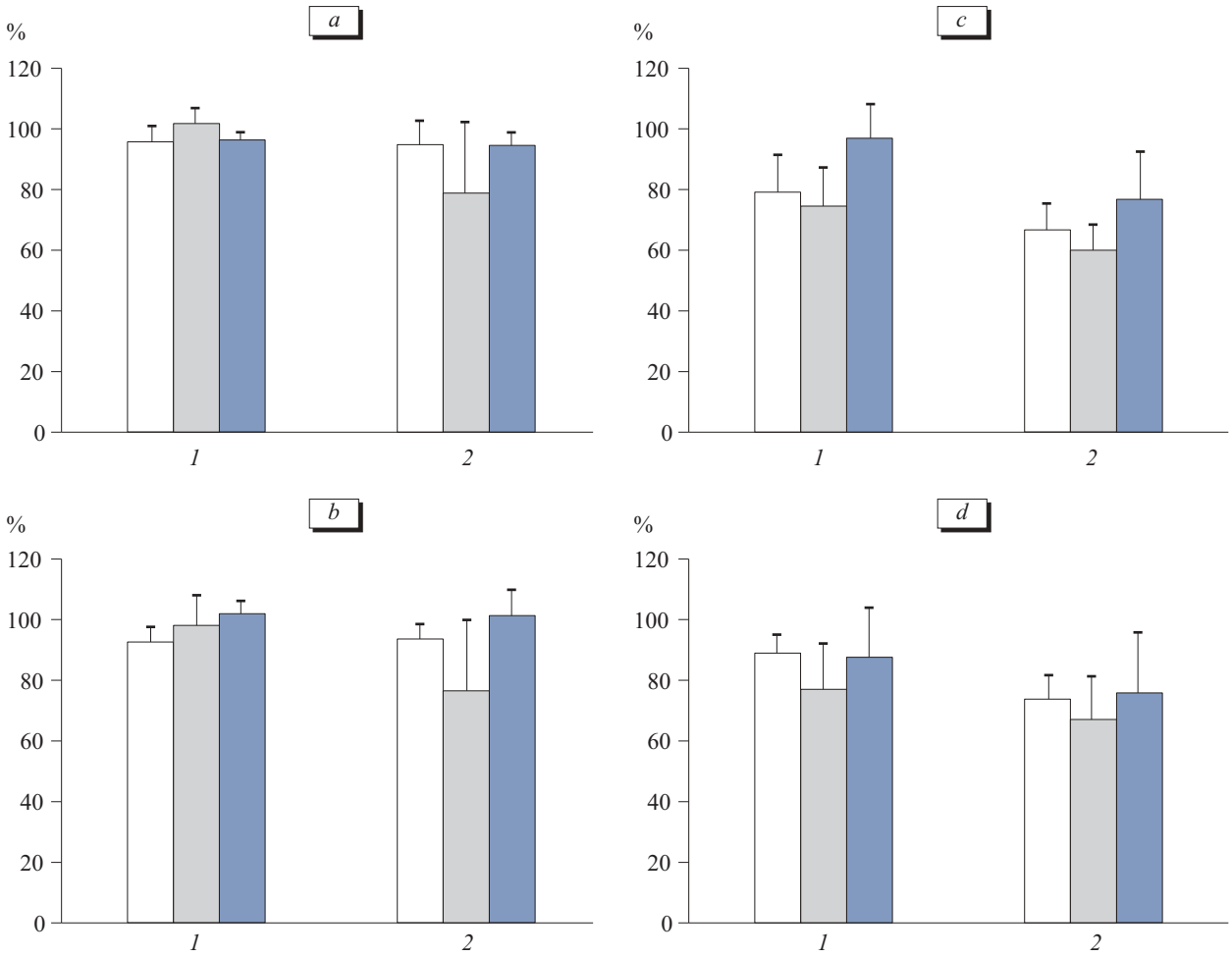

Fig. 1. Changes in the content of erythrocytes (*a*), hemoglobin concentration (*b*), total leukocyte count (*c*) and number of segmented neutrophils (*d*) in patients with stage III-IV lung cancer receiving antitumor chemotherapy by CAM (light bars) or CVC (gray bars) protocols and in patients with stage III-IV breast cancer receiving CAF protocol (blue bars). Here and on Fig. 2 and 3: abscissa: *1* and *2* — after 1st and 2nd course, respectively; ordinate: call count, % of initial value. Confidence intervals at $p<0.05$.

minimum values were observed in patients with lung cancer receiving CAM and CVC protocols (to 66.66 and 59.97% of the initial level, respectively, Fig. 1, *c*). The leukocyte count decreased primarily due to a decrease in the number of segmented neutrophils (more pronounced after the second course of chemotherapy, Fig. 1, *d*). It should be noted that most pronounced leukopenia was observed in patients with III-IV stage lung cancer receiving CAM protocol.

Analysis of sternal punction specimens revealed considerable devastation of the erythroid hemopoietic stem (Fig. 2, *c*, *d*) despite the absence of appreciable suppression of peripheral erythron elements under the effect of chemotherapy (Fig. 1, *a*, *b*). The most pronounced suppression was documented for the number immature erythrokaryocytes throughout the observation period. Comparative study showed that CVC and CAM protocols were most toxic (Fig. 2, *c*).

Morphologically discernible cells of the granulocytic hemopoietic stem demonstrated different reactions to chemotherapy protocols. In patients receiving CAM protocol the number of immature neutrophilic granulocytes (myeloclasts, promyelocytes, myelocytes, metamyelocytes) progressively decreased and attained 55.3% of the initial level after the second course. In contrast, compensatory changes in granulocytopoiesis were noted during CVC and CAF treatments: after the first course the content of immature neutrophilic granulocytes increased by 19.0 and 21.8%, respectively, compared to the levels before treatment (Fig. 2, *a*). We assume that CVC and CAF combinations do not cause considerable damage to granulomonocytic precursors capable of mitotic division, which creates the basis for activation of regeneration processes in the bone marrow at early terms of cytostatic disease [2,8]. However, reparative capacities of the granulocytic stem are exhausted at later stages. This assumption is confirmed by decreased content of immature neutrophilic granulocytes below the initial level after the second course of chemotherapy (Fig. 2, *a*). At the same time, in patients receiving CAF protocol com-

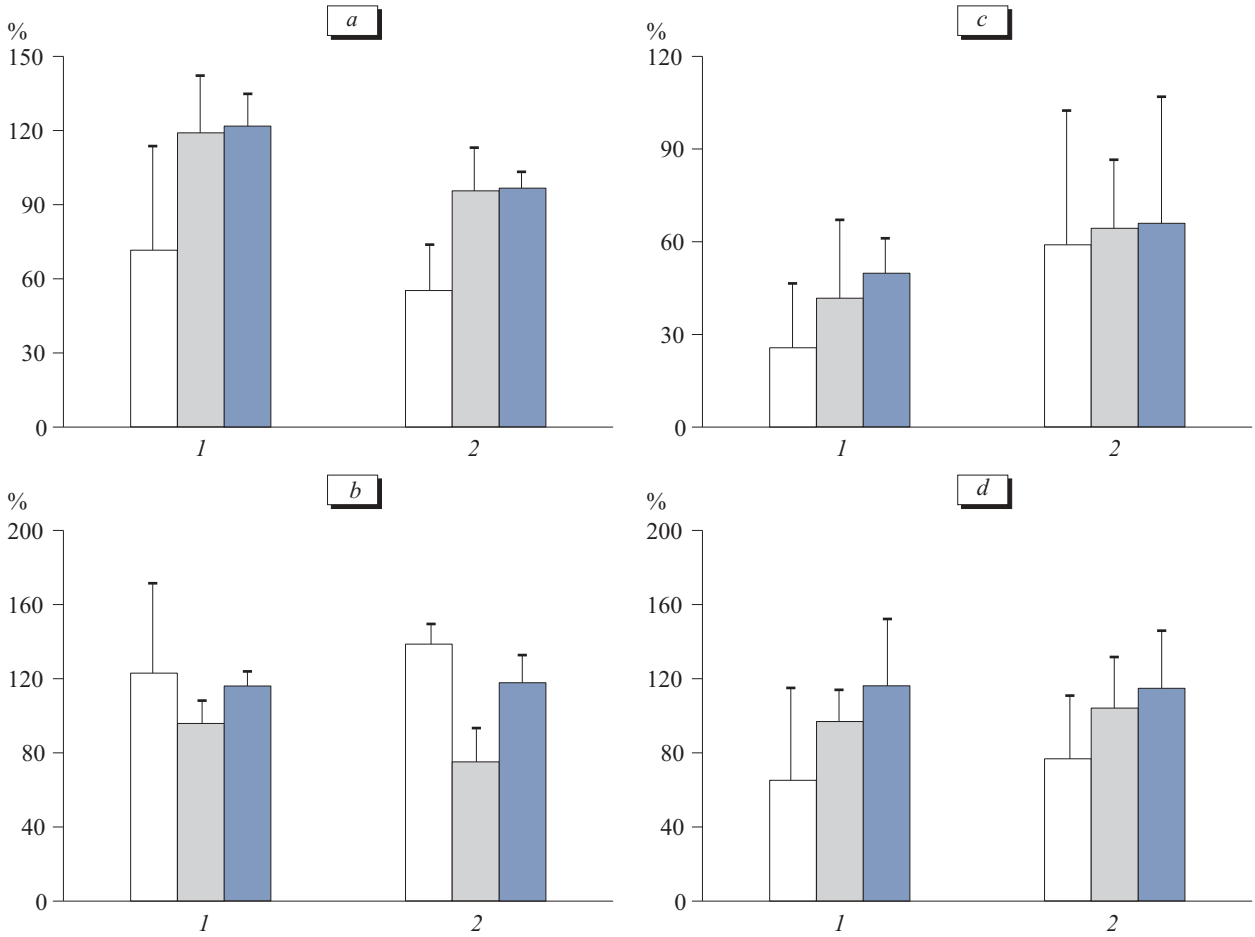

Fig. 2. Dynamics of the content of immature neutrophilic granulocytes (*a*), mature neutrophilic granulocytes (*b*), immature (*c*) and mature neutrophilic erythrokaryocytes (*d*) in the bone marrow of patients with stage III-IV lung cancer receiving antitumor chemotherapy by CAM (light bars) or CVC (gray bars) protocols and in patients with stage III-IV breast cancer receiving CAF protocol (blue bars).

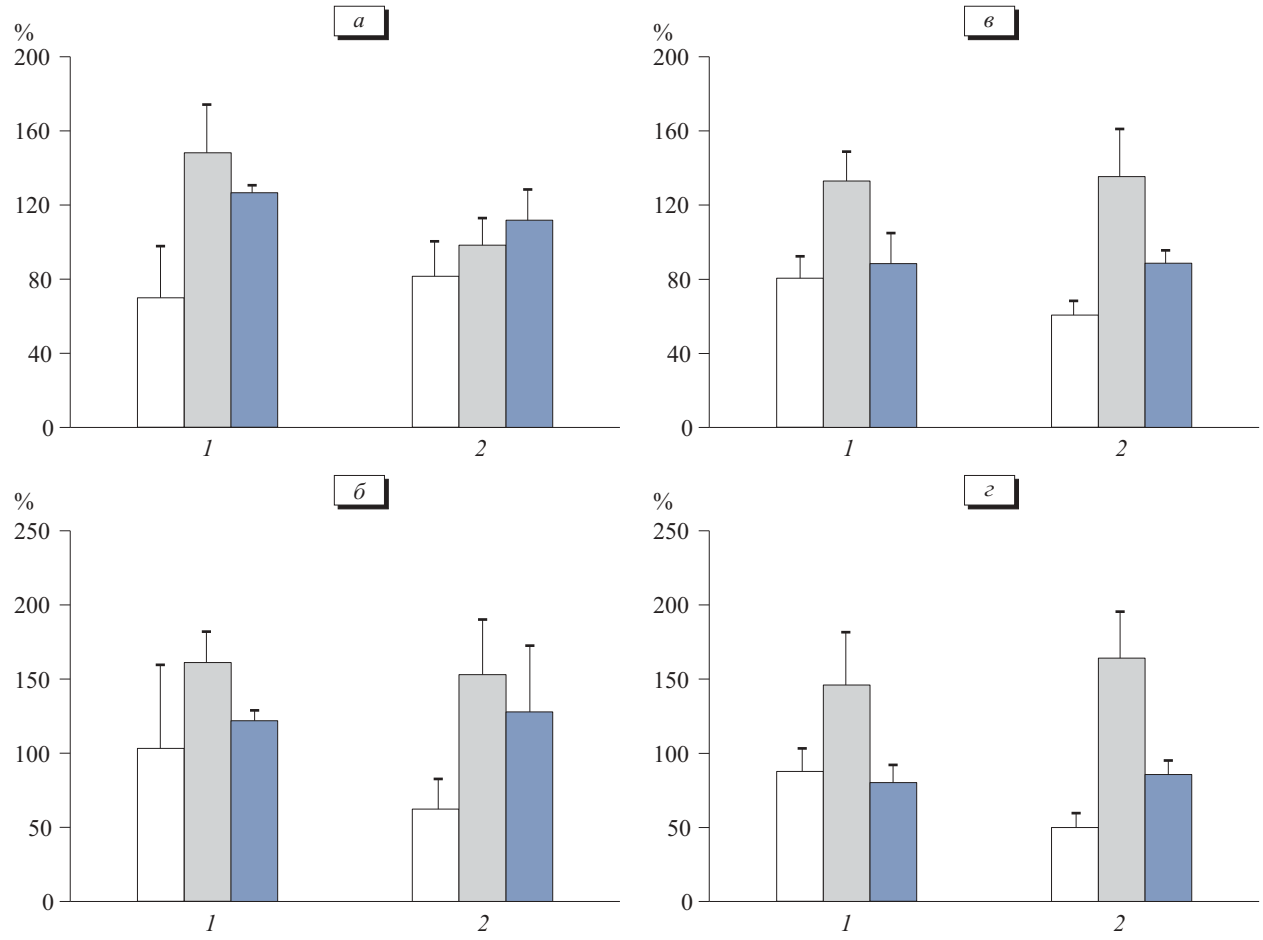

Fig. 3. Dynamics of the content of committed precursors of granulomonocytopoiesis (*a*), erythropoiesis (*b*) in the bone marrow and granulomonocytopoiesis (*c*) and erythropoiesis (*d*) in the peripheral blood of patients with stage III-IV lung cancer receiving antitumor chemotherapy by CAM (light bars) or CVC (gray bars) protocols and in patients with stage III-IV breast cancer receiving CAF protocol (blue bars).

pensatory shifts in the content of mature granulocytes were observed throughout the entire observation period (Fig. 2, *b*).

The role of accelerated maturation of committed hemopoietic precursors in hemopoiesis recovery after cytostatic myelosuppression is proven and can hardly be overestimated [2]. Therefore, we paid particular attention to these cells in the bone marrow and in circulation.

The use of CAM protocol reduced the content of erythro- and granulomonocytopoiesis precursors in the hemopoietic tissue throughout the entire observation period (Fig. 3, *a*, *b*).

Similar dynamics was observed for peripheral pool of hemopoietic precursors: the number of circulating CFU-E and CFU-GM progressively decreased in patients receiving CAM protocol (Fig. 3, *c*, *d*). Probably, these drugs damage not only proliferating morphologically identifiable hemopoietic cells, but also stem cells. At the same time, the absence of signs of the release of CFU-GM and CFU-E into circulation under conditions of CAM treatment can be related to disturbed mobilization of clonogenic elements and to exhaustion of their pool in the bone marrow.

When CVC and CAF protocols were used, we observed an increase in the number of bone marrow precursors of erythropoiesis by 61.1% (CVC) and 22% (CAF) and precursors of granulomonocytopoiesis by 48.1 (CVC) and 26.6% (CAF). These parameters remained increased after the second course of chemotherapy, although no significant differences from the initial level were detected (Fig. 3, *a*, *b*). The increase in colony-forming capacity of the bone marrow tissue is an obligatory component of the universal reaction of the hemopoiesis to extreme conditions (*e.g.* cytostatic trauma) [3]. The central stress-realizing structures via activation of neuroendocrine mechanisms acting directly on hemopoietic cells, or via elements of the hemo-

poiesis-inducing environment intensify proliferation and differentiation of hemopoietic cells.

Treatment by CAF protocol, despite stimulation of CFU-E and CFU-GM in the bone marrow, decreased the content of erythro- and granulocytic precursors in the peripheral blood after the first and second courses of chemotherapy. This is determined, along with redistribution reactions in the hemopoietic system, by disturbed mobilization of hemopoietic precursor cells into the peripheral blood [10].

Analysis of the content of erythro- and granulomonocytic precursors in the peripheral blood of patients with lung cancer receiving CVC protocol revealed considerable increase in these parameters (by 1.5 times compared to the initial level) throughout the entire observation period (Fig. 3, *b*, *d*). This is determined by pronounced activation of clonogenic elements in the bone marrow. Moreover, it is known that administration of antitumor drugs by short 2-day courses is most effective for mobilization of hemopoietic precursors into peripheral blood.

Thus, the use of all three protocols in patients with different localization of the tumor process showed high resistance of erythro- and granulomonocytopoietic precursors to the damaging effects of cytostatic drugs compared to morphologically differentiated bone marrow elements, which are the main target for chemotherapy. This agrees with the data that committed precursors, elements of the buffer compartment of the hemopoietic system, rather that polypotent hemopoietic stem cells are responsible for replenishment of the pool of morphologically identifiable hemopoietic cells under extreme conditions [2,3,5].

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