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Hematopoiesis Restoration After Autologous Bone Marrow Transplantation Using a Frozen Graft

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ABSTRACT

The springboard for hematopoiesis is the bone marrow located in the spongy bones. Hematopoiesis assessment is primarily carried out by assessing the ratio of hematopoietic, adipose and bone tissue. The ratio in the study is 1:0.8:0.5 as the norm. A hematopoietic area of less than one was assessed as a hypocellular biopsy. In 40 patients who underwent autologous bone marrow transplantation with cryo-frozen stem cells, 20 trepanobiopsies were examined within +30 days after autologous bone marrow transplantation. Ten trepanobiopsies were performed on patients with lymphomas and ten on patients with myeloma.

Keywords: hematopoiesis, autologous bone marrow transplantation, frozen transplant

Because bone density in patients with myeloma is very low and repeated trepanobioptates can lead to spontaneous pathological fractures of the pelvic bones, biopsies were performed only once during the period of bone marrow recovery on day 30 after autologous bone marrow transplantation. However, since all patients were available during the separate follow-up periods and the results of their biopsy studies made it possible to conclude the state of hematopoiesis after autologous bone marrow transplantation, it was decided to include these data in the analysis. Figure 1 shows the results obtained in these cases since they may reflect changes characteristic of the hematopoietic state of autologous bone marrow recipients in the post-transplant period.

From the data obtained, it can be seen that even after autologous bone marrow transplantation, the cellularity of the bone marrow in none of the patients reached average values on the 30th day. A decrease in cellularity occurred even at 1 month ($P<0.05$) due to the myelotoxic effect of the conditioning regimen, which suggests that it is too early to talk about complete bone marrow repair at +30 days.

In all patients with myeloma who underwent trepanobiopsy (n=10), a pronounced thinning of the bone beams due to smooth bone resorption was revealed. In 2 cases of multiple myeloma, bone neoplasm in the form of a focal osteoblastic reaction was noted. The medullary cavities were wide. The ratio between adipose bone

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marrow and myelokaryocytes varied from pronounced hypoplasia when only fatty bone marrow was detected in the cavities adjacent to the cortical layer, and myelokaryocytes were found in deep cavities (6 cases) to an approximately equal ratio of adipose and hematopoietic tissue (3 cases) or a slight predominance of the latter (2 cases) due to a large number of plasma cells scattered singly and in small groups among the elements of myelopoiesis. However, there were no mitotically dividing cells. All biopsies were characterized by hypocellularity.

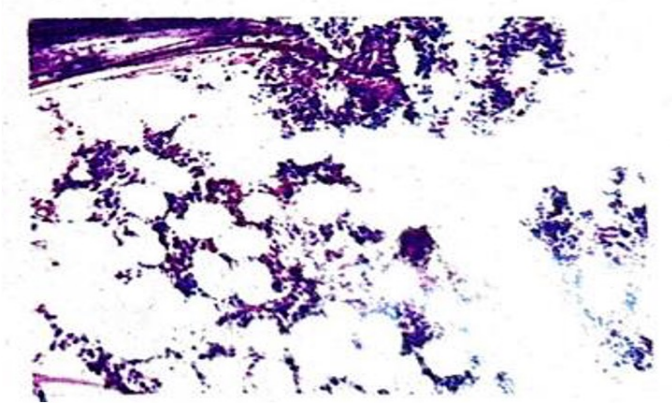

Figure 1. Trepanobioptate of patients with MM on day +30 after autologous bone marrow transplantation.

The distribution of lipocytes was uneven; they were located mainly along the bone beams. The erythroid sprout was reduced in 8 patients; in one case, it corresponded to the norm, and in another 1 case, it was dilated. Dysplastically altered forms were identified among erythrokaryocytes.

Myelokaryocytes lay mostly densely, but they were loose in several cavities. The cell composition determined the elements of all three hematopoiesis sprouts.

The main part of the cells were cells of the lymphocyte series. In 6 cases, it is dilated, narrowed in 1, and averaged in 3. The number of granulocyte cells was standard in 2 patients, which increased in 3 patients and remained normal in 5. It was represented mainly by mature and intermediate generations in approximately equal proportions; in some places near the bone beams, there were fields of immature granulocytes. The number of megakaryocytes in 3 cases corresponded to the norm, while in 5 cases, their number was significantly reduced. Individual dysplastically altered forms were identified among megakaryocytes: microgenerations, dystrophically altered cells, and holonuclear cells. Megakaryocytes were mainly located evenly singly near the sinuses, but there were cases with their uneven distribution. In 2 cases, expansion of the megakaryocytic sprout was noted.

In six cases, lymphatic and/or plasma cell infiltration was detected in the bone marrow: in two patients, this was due to the persisting infiltration by atypical plasma cells, and in four others, reactive lymphoid nodules were detected. In four cases, the number of lymphocytes and plasma cells did not exceed the norm.

Focal hemodynamic disorders, areas of coarsening and fibrosis, increased microvascular density, and sclerosis of the vascular wall were often found in the stroma. The hematopoietic and adipose tissue ratio was 0.7:1.0:0.5 and was assessed as hypoplastic. Dysplasias of erythrocytes and megakaryocytes were noted in the morphology of cells.

Qualitative analysis of hematopoietic tissue showed that in bone marrow biopsies obtained from patients with lymphomas (n=10) after transplantation, cells of three hematopoiesis sprouts were detected at all stages of maturation. In 8 patients, enlargement of the erythrocyte sprout was observed. Dysplastic changes in erythroid cells were found in half of the cases and were manifested by the presence of many erythroblasts and megaloblastoid forms. In one case, the number of megaloblastoid forms sharply increases. Mitoses were found among the cells, sometimes in pretty large numbers. As in the case of multiple myeloma, the central part of the granulocyte cells were mature cells. Narrowing of the granulocytic sprout was observed in 5 cases. In 2 cases, The granulocytic sprout corresponded to the norm, and in 3 cases, the granulocytic sprout was expanded due to mature and ripening forms, as shown in Figure 2.

The number of megakaryocytes in 3 cases was normal, while in the remaining 7 cases, their number was significantly reduced, as in patients with multiple myeloma. Among megakaryocytes, microgeneration and naked nuclei were revealed. Megakaryocytes were located unevenly throughout the trepanobiotate, more often in single specimens. In contrast to the group of patients with multiple myeloma, there were no cases of myelocaricitic growth enlargement.

In 8 cases, lymphocytic infiltration was detected in the bone marrow: in 2 patients, this was due to the persistence of lymphocyte infiltration since autologous bone marrow transplantation was performed for an

incomplete metabolic response, and in the remaining six patients, it was probably due to a delay in the recovery of the granulocyte germ and was relative. In 2 cases, the number of lymphocytes did not exceed the norm.

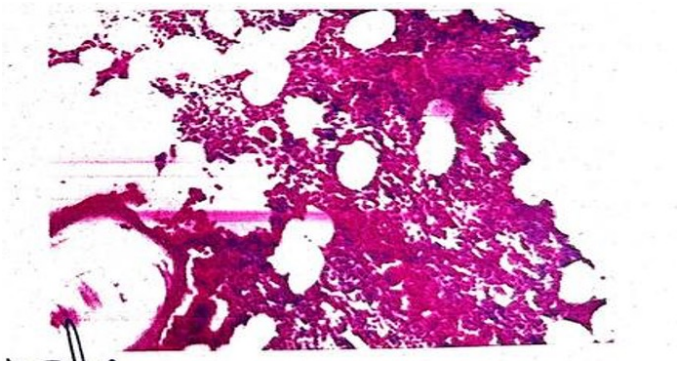

Figure 2. Trepanobioptate of patients with lymphomas on +30 days after autologous bone marrow transplantation.

In the stroma, as well as in cases with multiple myeloma, focal hemodynamic disorders, areas of fibrosis, increased microvascular density and sclerosis of the vascular wall were revealed. However, the ratio of hematopoietic tissue to adipose and bone tissue was 0.8:1.0:0.5. Although hematopoiesis was dysplastic, it remained cellular.

A decrease in the area of hematopoietic bone marrow in patients using a cryo-frozen transplant is most likely a consequence of the slow proliferation of stem cells, the influence of hyperthermia in febrile fever, and infectious complications.

Myelotoxic agranulocytosis is a natural stage of bone marrow transplantation caused by myelosuppression after the use of a cytostatic drug at the time of conditioning designed to eradicate the tumour clone and, as a side effect, inhibits all sprouts of bone marrow hematopoiesis. After conditioning, bone marrow hematopoiesis undergoes irreversible myelosuppression and can recover only after introducing a suspension of the graft. Hematopoiesis is considered to be restored when leukocytes, erythrocytes and platelets reach average values, and the exit from myelotoxic agranulocytosis is considered to be the achievement of an absolute number of leukocytes of more than 1000, and neutrophils more than 500 per μl. In this case, the 1st of 3 consecutive days of recovery of indicators is stated.

Neutropenia is divided into 3 degrees of severity: mild, moderate, and severe. In the study, patients were mostly severely neutropenic because they were on a myeloablative course of conditioning.

To assess the restoration of hematopoiesis in Group I patients, morphological studies of the peripheral blood of all 40 patients were performed at +7, +14, +21, +30, +60 days after autologous bone marrow transplantation. Although peripheral blood analysis was taken daily during the recovery period, the study included data only from the specified periods since there were no more or less significant fluctuations in the dynamics of daily indicators. The number of leukocytes, erythrocytes, and platelets in the peripheral blood and their morphological characteristics were assessed.

Myelotoxic agranulocytosis in this group of patients developed on +3 days on average (from -1 to +5 days) and lasted on average 17 (from 15 to 26 days). A slight leukocyte rise began by the 15th day after autologous bone marrow transplantation. The first day of leukocyte recovery was close to day 21 (from 18 to 25 days) L-1.2±1.0 (p=0.0003). At the same time, the data analysis revealed that the rise of leukocytes is not uniform but a wave-like line with periods of rise and decline after 2-3 days, then a constant increase in granulocytes is shown in Figure 6. Such fluctuations can be explained by damage to the transplant during freezing, thawing, or exposure to chemotherapies during numerous courses of chemotherapy.

With the development of severe neutropenia, especially with prolonged myelotoxic agranulocytosis, the risk of developing infectious complications increases when the neutrophil level is less than 500/μL. At the same time, 21 (52%) patients developed febrile neutropenia, 18 (45%) infectious complications, and only 1 (3%) patients had a period of myelotoxic agranulocytosis without complications.

At the same time, all patients from the +7th day were prescribed G-CSF at a dose of 300 IU subcutaneously once a day. Among the infectious complications, the most common were enteropathy (diarrhoea) in 11 (27%), 3 (9%) pneumonia, 3 (9%) paraproctitis, and 1 (3%) pulmonary intestinal sepsis.

The most common infectious agents were E. coli, Staphylococcus aureus, and Klebsiella pneumonia; the pathogens were sensitive to many antibacterial drugs, and treating infectious complications did not present much difficulty. Also, 34 (85%) patients in this group were diagnosed with acute cytomegalovirus infection, manifested by diarrhoea, pneumonia and lymphadenopathy. Taking this into account, IVIG was added to the transplantation protocol along with antiviral drugs +1 day at a dose of 1 g/kg/day once.

Thrombocytopenia is the second important complication in bone marrow transplantation. The number of megakaryocytes after transplantation naturally decreases after conditioning and is restored with the beginning of the repair of the "new" bone marrow. In the study, the presented changes in the state of bone marrow hematopoiesis demonstrate different recovery times for each cell line after myelosuppressive exposure to myeloablative conditioning courses. A sign of platelet growth recovery is the first of three consecutive days of stable hematopoiesis, in which the platelet level is more than 20,000.

The number of megakaryocytes in patients with multiple myeloma and patients with lymphomas is reduced by 50% and 70%, respectively. At the same time, in patients with multiple myeloma, biopsy specimens, the bone marrow remains hypocellular, and megakaryocytes are located in depressions around the trabeculae, in some places clusters, and in patients with lymphomas +30 days, cellularity in 70% of patients is expected. However, there are few megakaryocytes located in single elements throughout the biopsy.

The myelogram data (bone marrow aspirate) of megakaryocytes were sufficient but were reduced in size, and there were platelet-free and naked nuclei. Significant megakaryocyte dysplasia was noted, and the ratio of normal megakaryocytes to dysplastic ones was 1:3.5.

According to the severity, thrombocytopenia is divided into 3 degrees: mild, moderate and severe. As with neutropenia, in the study, patients were mostly severely thrombocytopenic, as they received high doses of cytotoxic drugs. The platelet level after bone marrow conditioning was 0, while a high risk of hemorrhagic complications naturally developed.

The platelet count of Group I patients was carried out according to the morphological examination of the peripheral blood of all 40 patients at +7, +14, +21, +30, +60 days after autologous bone marrow transplantation.

In our study in group IA, engraftment of platelets with transfusion independence (i.e. platelets above 30 thousand) was observed in 38 (94%) patients. This patient received platelet replacement transfusion 2 times a week. However, at 21 weeks after autologous bone marrow transplantation, the patient developed hematuria; the patient received thromboconcentrate therapy, and bleeding from the urinary tract did not stop, which led to bladder tamponade. Emergency therapy was carried out, and a nephrostomy was installed, but after 2 weeks, the patient died from uncontrolled bleeding. The cause of death was intra-abdominal bleeding due to a rupture of the bladder. Another 1 (3%) patient in the same group had unilinear graft failure; the platelet level did not rise above 20±10 thousand on +30 and +60 days, but the patient did not need platelet transfusion and thrombopoietin due to the absence of hemorrhagic complications. It is worth noting that the use of thrombopoietin, even in high doses, did not lead to a significant increase in platelets. In the bone marrow biopsy on day +30, hypocellularity, predominance of adipose bone marrow, erythrocyte dysplasia, and megakaryocytes were not found in the bone marrow aspirate. Both patients were diagnosed with multiple myeloma.

Thrombocytopenia in this group of patients developed on +3 days on average (from -1 to +5 days) and lasted on average 16 (from 14 to 24 days). A slight platelet rise began by the 16th day after autologous bone marrow transplantation. On the first day of stable platelet recovery, they approached day 18 (from days 14 to 26). At the same time, the increase in platelets is logarithmic. When analyzing the data, it was found that platelets were at the level of low numbers; then, with the restoration of hematopoiesis, they multiplied to standard numbers in just 2-4 days. For example, on the 15th day of therapy, the patient's platelet level remained ± 5 thousand; on the 16th day, there was a 2-fold increase to 10 thousand; on the 17th day, the platelet level could already be 20-25 thousand. At the same time, most patients remained independent of platelet transfusion; the dynamics of platelet rise are shown in Figure 7. Such a delay can be explained by the peculiarity of platelet growth recovery when using a frozen transplant or the effect of chemoprerapates during numerous courses of chemotherapy. At the same time, there was no significant difference between patients with multiple myeloma and lymphomas. This allows us to conclude that the difference between the conditioning courses does not significantly affect the restoration of the megakaryocytic sprout. The platelet recovery curve tended to grow steadily, with no critical falls. In 2 (6%) cases, after the 28th day, patients were treated with thrombopoietin (L-thrombosis) at 100 mcg - 2 times a day since isolated platelet germ insufficiency was diagnosed.

Patients with long-term thrombocytopenia naturally increase the risk of hemorrhagic complications. Having studied the data of domestic and foreign literature, as well as according to the recommendations of EBMT during autologous bone marrow transplantation, we adhered to the tactics of replacement therapy to maintain the platelet level of at least 30 thousand/μl to reduce the

risk of bleeding. Life-threatening: cutaneous hemorrhagic in the form of small petechiae in 14 (35%), nosebleeds 4 (10%), gingival bleeding 4 (10%), hemorrhoidal bleeding 4 (10%), uterine bleeding 2 (6%), are presented in Diagram 2. These complications were quickly controlled and did not exceed the percentage described in the literature.

The erythrocyte sprout is the most persistent in the hematopoietic system. Despite the use of high-dose chemotherapy in autologous bone marrow transplantation, the development of anaemia is planned, making it possible to correct hypoxia adequately. This is most likely due to the morphology of erythrocytes, in which cytotoxic drugs mainly act on nucleated cells, and the lifespan of the erythrocyte is 100-120 days. Thus, the red blood cells circulate in the blood function long, even after the patient has developed myelotoxic agranulocytosis. Anaemia requiring replacement therapy is considered to be the level of haemoglobin <70 g/L. Although haemoglobin restoration is not a classic criterion for engraftment, it is impossible to talk about normal bone marrow function without stable recovery of red blood cells. So, the day of engraftment of erythrocyte growth is considered the 1st out of 7 consecutive days of stable hematopoiesis, at which the haemoglobin level is above 70 g/l without transfusion support. In the study, 100% of Group I patients developed anaemia. Of these, 24 (60%) have severe anaemia, and 16 (40%) have moderate anaemia.

At the same time, transfusion support was used only in the development of severe anaemia with clinical manifestations (dyspnea, tachycardia, hypoxic complaints). Erythrocyte transfusion was performed in 4m (10%) patients in a volume of more than four doses; these were patients who developed hemorrhagic complications in the form of nasal and uterine bleeding, 10 (25%) transfused 3-4 doses and 10 (25%) patients required transfusion from 1 to 3 doses of erythrocyte mass. 12 (40%) patients did not require erythrocyte replacement therapy at all. In these patients, the haemoglobin level was about 80 ±8 g/l, but no anaemic complaints were observed, and there were no clinical manifestations of hypoxia.

Bone marrow biopsy, myelogram, and peripheral blood analysis assessed the recovery of the erythroid sprout. The study found that during the period of hematopoiesis recovery, all myelogenous and erythroid series elements have dysplastic elements.

Conflict of interest - The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Financing – No financial support has been provided for this work.

Data availability statement - The original contributions presented in the study are included in the article material, further inquiries can be directed to the corresponding authors.

Ethics approval and consent to participate - All patients gave written informed permission to participate in the study.

Consent for publication - The study is valid, and recognition by the organisation is not required. The authors agree to open the publication.

Availability of data and material - Available

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