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Assessment of the Morphological Picture of Bone Marrow and Peripheral Blood in the Early Days after Allogeneic Bone Marrow Transplantation

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Abstract Hemablastosis is one of the most acute scientific problem in medicine. Bone marrow transplantation is currently a reliable method of tumor removal in leukemia. Although the story of transplantation is more than 50 years, the issues of bone marrow restore after allogeneic and autologous transplantation want in addition analyzing, which is the relevance of this topic. Objective: To observe the morphological picture of the bone marrow based on the results of ilium biopsies in patients after allogeneic bone marrow transplantation in the early post-transplantation period. Materials and Methods: A morphological evaluation of bone marrow biopsies from 30 patients after autologous bone marrow transplantation performed at RSSPMCH from 2023 to 2024. Results: From the facts obtained, it is obvious that after bone marrow transplantation, the cellularity of the bone marrow in none of the patients reached normal values even through the 30th day. In 22 (74%) patients, the bone marrow riches normal cellularity by day 60. In 8 (26%) cases, diagnosed hypocellularity in the bone marrow. The ratio of adipose bone marrow to myelokaryocytes was 0.5:0.9, indicating hematopoietic hypoplasia. A poor cellularity was persisted in 1/3 of patients within 60 days, which manifested as triple cytopenia in 7 (23%) patients. Conclusion: Based on these findings, we finish that despite highly stable peripheral blood values, it's far still too early to talk about completly engraftment of bone marrow stem cells, even via day 60.

Keywords Bone marrow allotransplantation, Stem cells, trepanobiopsy, Hematopoietic restoration

1. Relevance

Hemablastosis is one of the most acute medical issues these days. The development of pharmaceuticals and targeted drugs have progressed the results of chemotherapy. However, bone marrow transplantation (BMT) stays a reliable method for eradicating the tumor clone in leukemia [11]. Developing techniques in medicine, especially the appearance of the cells separators, have made bone marrow transplantations much less traumatic because of using cells from peripheral blood. High-dose chemotherapy accompanied by stem cell transplantation has made patients relapse-free survival upper than 30% [2]. However, the main results of treatment depends of the type of leukemia, on the timing of transplantation and the degree of the ailment. Molecular genetic analysis is also important. Cytogenetic strategies now play a unique role in predicting the final results of the disorder [1]. Allogeneic bone marrow transplantation surpasses the results received with polychemotherapy and using targeted drugs. The disease-free survival in patients who received allogeneic bone marrow transplantation during complete remission is 45-75%, and they relapse no greater than 15%. Therefore, in all patients with a match donor (HLA-equal),

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allogeneic bone marrow transplantation is an indisputable recommendation [2]. However, finding HLA-match donors is a hard assignment, and only 30% of patients may also have a related, appropriate sibling donor. In cases when no donor, patients can undergo myeloablative chemotherapy and support recovering hematopoiesis with an autologous transplant [3,5]. And in these cases, there may be no graft-versus-leukemia effect, and there is additionally a threat of contaminating the stem cells with tumor clones, which increases the likelihood of relaps of patients [7].

Although the history of transplantation is more than 50 years, the issues of bone marrow repair after allogeneic and autologous transplantation stay poorly understood [8]. It is so few studies dedicated on the timing of the restoration of bone morrow parameters after transplantation and the morphological patterns of hematopoiesis. A limited number of publications about the character of hematopoiesis after allogeneic bone marrow transplantation based on bone trepan biopsies [5]. At the same time, it is obvious that bone marrow morphology data is needed to compare the picture of peripheral blood, and it is important to evaluate not only the number but also the quality of cells in the bone marrow [10]. The comparison of peripheral blood parameters and bone marrow cell morphology determines the relevance of this work.

Study Aim: to study the morphological picture of bone marrow according to trepan biopsies in patients after allogeneic bone marrow transplantation during the early period.

2. Materials and Methods

The study included 30 patients with acute leukemia who were treated at the Republican Specialized Scientific and Practical Medical Center of Hematology in Uzbekistan (RSSPMCH) in the transplantation department from 2023 to 2024. 30 bioptates were analyzed on day +30 and on day +60 after alloBMT. Trepan biopsy was performed under local anesthesia from the posterior spine of the ilium. The staining of the preparations was carried out with hematoxylin -eosin, peripheral blood smears - by Romanovsky - Giemse. Statistical processing of the results was carried out in the Statistica 10.0 program for Microsoft. Conditioning before bone marrow transplantation was performed according to the low-intensity regime (RIC) FLUMEL protocol, which consisted of fludarabine 30 mg/m^2 for 6 days, melfolan 140 mg/m^2 on 2 consecutive days -3 and -2 of alloBMT. In cases with a 100% HLA-identical donor, chemoprophylaxis of the graft-versus-host reaction (GVHD) was performed by administration on +3 and +4 days of Cyclophosphamide at a dose of 25 mg/m2 simultaneously with the antidote Uromethoxane (mesna) in a ratio of 1:1.5. In haploidentical bone marrow transplantation (when HLA match is less than 9:10, but not less than 5:10), GVHD prevention began 15 days before transplantation. On day 15, the administration of Mycophenolate mofetil 30 mg/kg (but not more than 2 g/day) and Rituximab at a dose of 375mg/ m^2 began. Cyclophosphamide was also administered on the same days and at the same dose as with alloBMT with a full match donor. On day 5, in both cases, Cyclosporine A was added intravenously at a dose of 3 mg/kg, then this drug continued until day 100 after alloBMT. All patients 30 (100%) in the study received native (non-frozen) peripheral blood stem cells from related donors. The average cell count of the transfused graft was 6.03±1.5*10^9/kg of cells (4.5-7.45*10^9 /kg) p<0.0001.

3. Results

Table 1. Characteristics of patient in study group

	Study group (n=30) N %	
Age, median (range)	46 (19 – 60)	
Sex		
Male	20	70
Female	10	30
Diagnosis		
AML	18	60
ALL	8	26.6
AA	4	13.3
Number of HSCT		
First allo-HSCT	30	100
Donor		
Haploidentical	15	53.1
MMRD	2	6.2
MRD	13	40.7
Stem cell source PBSC	30	100
Conditioning regimen		
RIC	30	100
Post-transplant cyclophosphamide GVHD prophylaxis	30	100

Of the 30 patients who underwent allogeneic bone marrow transplantation (alloBMT) from related donors, 60 trepan biopsies were performed and 60 were analyzed. A biopsy of the ilium was performed at +30 and +60 days after alloBMT. The group consisted of patients with AML-18(60%), ALL- 8(26.6%) and aplastic anemia 4 (13.3%). The average age of the patients was 46 (19-60), gender separation was male -20 (70%), female – 10 (30%). All patients in the study group received their first transplant – 30 (100%). Peripheral blood was used as a source of stem cells in all 30 (100%) patients. At the same time, there were 13(40.7%) full matches related, haploidentical 15(53.1%) and partial match 2(6.2%) donors. The characteristics of the patients are shown in Table 1.

According to the standards of patient management, after alloBMT biopsy of bone with a study of bone marrow hematopoiesis, it should be performed within +30, +60, +90 days, then once every 6 months for the next 2 years. Our study includes data from the only first 2 checkpoints. In all

patients who underwent histological analysis of bone (n=30), thinning of bone beams was revealed, but without signs of resorption. There was a greater violation of the microarchitecture of bone tissue vessels in these samples. 22 (74%) had normocellular BM by day +60. Significant hypocellularity of BM was noted in 8 (26%) cases. The ratio between fatty bone marrow and myelokaryocytes prevailed towards adipose tissue was 0.5:0.9, which indicates hypoplasia of hematopoiesis. Lacunar spaces were replaced by adipose tissue, lipocytes were also located unevenly, mainly along bone beams. Hematopoietic cells in these patients were found in small numbers, sometimes in small clusters, and mainly consisted of lymphocytes. And myelokaryocytes were found in isolated quantities, no myelokaryocytes were found in 2 (6%) cases. The rest 28 (94%) myelocaryocytes were loose, scattered around the drug in places. 5 (17%) cells showed signs of dysplasia, and there were very few megakaryocytes in these biopsies, 1-2 per micro-preparation.

There were no mitotically dividing cells. Erythroid sprout was reduced in 2(6%) patients and was normal in 10(33%) cases. The bone marrow cavities were wide. The morphological picture of the bone marrow from the trepan biopsy is shown in Figure 1.

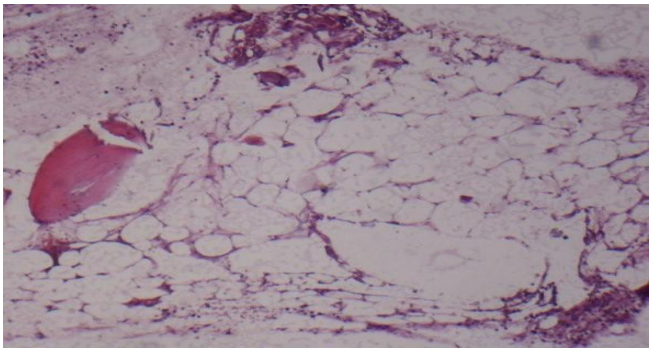

Figure 1. Histological picture of bone morrow at +30 day after allogeneic bone marrow transplantation

Fat cells were scattered unevenly, and it prevailed over the hematopoietic (Table 2).

The elements of all three hematopoiesis sprouts were determined in the cellular composition.

The number of granulocyte cells was normal in 7 (23%) patients and in 5 (16%) it remained reduced by + 30 days of alloBMT. The granulocyte sprout was represented mainly by mature and intermediate generations, in approximately equal proportions, with young and immature granulocytes occurring in places in the biopsy. The number of megakaryocytes in 15 patients (50%) was near to the norm, whereas in 15 (50%) cases their number was significantly reduced. Dysplastic, altered forms were detected among megakaryocytes: micro and macrogenerations, dystrophically altered, as well as naked nuclear cells. Megakaryocytes were located mostly evenly in several pieces near the sinuses, however, in 4 (13%) preparations there were cases with clusters of megakaryocytes near the bone beams. There was no expansion of the megakaryocytic germ in the study group.

Table 2. Characteristics of bone marrow hematopoiesis in patients with acute leukemia using native bone marrow at +30 and +60 days after allogeneic bone marrow transplantation

Indicators (n=30)		(Number of patients/ %) +30 day	(Number of patients/%) +60 day
Bone marrow cellularity	Hypocellular	8 (26%)	7 (23%)
	Normocellular	22 (74%)	23(7%)
	Hypercellular	0 (0%)	0 (0%)
Erythroid sprout	Narrowed	2 (6%)	2 (6%)
	Normal	28 (94%)	28(94%)
	Expanded	0 (0%)	0 (0%)
Granulocyte sprout	Narrowed	5 (16%)	4 (13%)
	norm	25 (84%)	26 (87%)
	Expanded	0 (0%)	0 (0%)
Megakaryocyte count	Reduced	15(50%)	15(50%)
	Normal	15(50%)	15 (50%)
	Increased	0 (0%)	0 (0%)
Lymphoplasmacytic sprout	Narrowed	0 (0%)	0 (0%)
	norm	28(94%)	30(100%)
	Expanded	2 (6%)	0(0%)
Cell dysplasia		5 (41%)	2 (6%)

In 2 (16%) cases, lymphoproliferation was detected in the bone marrow, as the granulocytic germ was reduced, in 10 patients the normal lymphocytic germ.

Focal hemodynamic disorders, areas of coarsening and sclerosis of the vascular wall, and vascular microarchitectonics were often found in the stroma. The ratio of hematopoietic tissue to fat and bone was 0.56:0.9:0.52 and was assessed as hypoplastic (Table 3).

Table 3. The morphological ratio of hematopoietic, adipose and bone tissue in the analysis of biopsies in patients with acute leukaemia on day 30

Indicators	Results of morphological study of trepanobiopsies
	Group alloBMT
	Acute leukaemia (n=30)
Hematopoietic tissue, %. M + m spread	25,6 ±1,13 (35,4-50,1) (p<0.0003)
Adipose tissue, %. M + m spread	44,0 ±0,95 (38,2-46,0) (p<0.005)
Bone tissue, %. M + m spread	23,3 ±0,62 (11,9-23,9) (p<0.0001)
correlation	0,56: 0,9:0,52

In cell morphology, dysplasia of erythrocytes and megakaryocytes was noted as described above. As can be seen from the table, hematopoiesis in patients in this group cannot be described as fully restored on day +30 after alloBMT, and hypocellularity persists in one third of patients as far as the results of biopsies allow. The area of the hematopoietic bone marrow is reduced, and the cells themselves, even in the case of normocellularity, show signs of dysplasia.

When analyzing the biopsy data on +60 days after alloBMT, it was noted that bone marrow cellularity was restored in 23(77%) of patients, but remained hypoplastic in 7(23%). In our study, this meant that every 3th of the patients had incomplete bone marrow recovery by the end of the 2nd month of follow-up (figure 2).

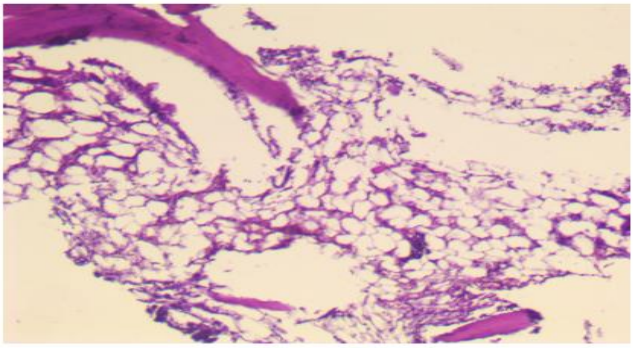

Figure 2. Histological picture of bone morrow at +60 days after allogeneic bone marrow transplantation

During this period, the percentage of dysplastic cells decreases significantly, but remains at 2(6%). Granulocytes recover by day 60 in 25(87%) of patients and erythrocytes in 28 (94%) of patients. The lymphocytic germ is restored in 30 (100%) of patients by the 2nd month of follow-up. However, megakaryocytic outgrowth is reduced in 50% of patients, even though there are enough platelets in the peripheral blood and the patient is transfusion independent. As can be seen from the table, in almost half of the patients, bone marrow hypocellularity persists even 60 days after transplantation.

To assess the recovery of blood parameters, morphological analyses of peripheral blood of all 30 patients were performed along with a biopsy on the +7,+14,+21, +30, +60 day after alloBMT, the results are shown in Table 4.

Although peripheral blood was analyzed daily during the recovery period, the study included data from only the specified dates, since there were no more or less significant changes in the dynamics of daily indicators. The number of leukocytes, erythrocytes, and platelets in peripheral blood was assessed, as well as their morphological characteristics.

Myelotoxic agranulocytosis (MTA) in this group of patients developed already before transplantation -2 days on average (from -3 to 0 days) and lasted on average 24 (from 14 to 58 days). A slight increase in leukocytes began already by the 16th day after alloBMT, only in some patients. The first day of leukocyte recovery was approaching day 20 (from day 16 to 25) WBC is 1.0± 0.5 (p=0.0003). At the same time, it was found that the rise of leukocytes in this group is not evenly, but an The wavy line with periods of rise and fall after 2-3 days, as was the case in the group with the use of a frozen transplant. This behavior of leukocytes in the group can be explained by the fact that in the group with alloBMT mainly patients with acute leukemia who received multicomponent chemotherapy in the induction course of therapy [5].

The first day of platelet recovery with transfusion independence occurred closer to day 17±2 (i.e., platelets above 20 thousand/ml were in 48 (64%) patients). In 10 (13%) patients, platelet recovery to safe numbers (at least 20 thousand/ml) occurred on day 17. Insufficiency of graft engraftment was observed in 4 (12%) patients. In 16 (53%), platelet recovery above 20 thousand/ml occurred by day 19±1 (p=0.003). By the 3rd week after transplantation, the platelet count was more than 50 thousand/ml in the majority, i.e. in 26 (90%) patients. +30, +60 days peripheral blood tests showed that platelets reached 100±30 thousand (p<0.003) and 180±50 (p<0.003) accordingly, this indicates the good functioning of the platelet germ. The thrombopoiesis stimulator, thrombopoietin, was not used in the group. The hemoglobin level and the number of red blood cells in the peripheral blood were evaluated when assessing the restoration of the red sprout. By day 14, the majority of patients 25(83%) were transfusion independent and the hemoglobin and erythrocyte levels were 80±5 g/l (p=0.0003) and 1.9±0.5 (p=0.001), respectively.

Table 4. Dynamics of peripheral blood parameters in group after allogeneic bone marrow transplantation

Indicators (n=30)	+7	+14	+21	+30	+60
Leukocytes (thousand)	0.00±0.01 (p=0.0003)	0.9±0.5 (p=0.0003)	1.5±1.0 (p=0.0003)	2.2±1.5 (p=0.0003)	3.3±1.1 (p=0.0003)
Neutrophilia (thousand)	0.00	0.5±0.1 (p=0.0005)	0.9±0.2 (p=0.0005)	0.9±0.7 (p=0.0005)	1.1±0.4 (p=0.0005)
Platelets (thousand)	0±1 (p=0.003)	5±2 (p=0.003)	50±10 (p=0.003)	60±30 (p=0.003)	100±30 (p=0.003)
Erythrocytes (million)	1.5±0.7 (p=0.001)	1.9±0.5 (p=0.001)	2.8±1.0 (p=0.001)	2.5±0.5 (p=0.001)	2.6±1.1 (p=0.001)
Hb (g/l)	68±10 (p=0.0003)	80±5 (p=0.0003)	90±10 (p=0.0003)	100±12 (p=0.0003)	100±15 (p=0.0003)

4. Conclusions

As can be seen from the study, as early as day 21, the hemogram indicators are approaching the values that allow the patient to dispense with replacement therapy with blood components. During this period, patients with peripheral blood tests have stable indicators and have often already been discharged from the hospital. With a more detailed analysis of bone tissue, 1/3 of patients after alloBMT had cell dysplasticity on +30 and even on +60 days after BMT. Not all patients from the observation group had graft engraftment. Bone marrow hypocellularity was diagnosed in 7 (23%) patients, which manifested itself in the peripheral blood of 4 of them as 3 line cytopenia, 3 like isolated red cell insufficiency. From all this, it can be concluded that despite the relatively stable peripheral blood levels, even at +60 days, it is still too early to talk about complete bone marrow repair, and it is advisable to leave patients under outpatient supervision.

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