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Fetal Stem Cells Use in Complex Treatment of Patients with Acquired Aplastic Anemia and Therapy Effect on Recovery of Hematopoietic Functions

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Abstract: The principal objective was studying effects of complex treatment using fetal stem cells (FSCs) on recovery of hematopoietic function. 50 patients suffering from aplastic anemia of different disease severity were under study and underwent complex treatment by use of cryopreserved suspensions containing fetal liver cells extracted from 7-12 week gestation fetuses. During the above study we proved effectiveness of FSCs use for the patients with acquired aplastic anemia (AAA). Positive effects on patient's subjective assessment of his/her condition along with an objective increase of tolerance to physical exercises were reported as early as during the first week after FSCs transplantation. We also recorded a stabilization of erythrocytes count, hemoglobin levels, platelets and leukocytes values in all patients under study. Reverse of infectious, hemorrhagic and anemic syndromes was achieved after treatment by use of FSCs. Significantly high erythrocytes and leucocytes counts controlled by laboratory tests together with reverse of anemia, hemorrhagic signs and infectious syndromes in the patients were also remarkable over 14 days after treatment. Within 7 days the patients felt improvement of their general state and increased tolerance to physical exercises. It has been proved that complex treatment by use of FSCs along with conventional therapy leads to stabilization of laboratory blood parameters and improved life quality among the patients with aplastic anemia.

Keywords: Acquired Aplastic Anemia, Fetal Stem Cells, Recovery of Blood Hematopoietic Function, Myelodysplasia

1. Introduction

Aplastic anemia is rare and potentially life-threatening breakdown of hematopoiesis which is characterized by pancytopenia and multicellularity of bone marrow (BM) [1]. In case of such pathology BM is hypocellular; however, commonly the patients do not reveal pathological infiltrate and increased range of reticulin [2]. Morbidity rates in case of aplastic anemia worldwide constitute up to 10 cases per one million of population annually. Conducted epidemiology studies established uneven frequency of such pathological incidence throughout different regions. Thus, in Far East countries the prevalence of aplastic anemia is twofold higher than in the countries of Europe and America [3]. Aplastic anemia is referred to hematopoietic diseases – myelodysplastic syndromes in particular, which inhibits or interrupts growth and maturation of all three hematopoietic lineages. Factors of aplastic anemia genesis could be associated with effects of ionizing radiation, impact of chemicals – mainly salts of aurum, arsenic, benzol; or

influence of medicines: chloramphenicol, phenylbutazone, chlorpromazine, antimetabolites or alkalizing substances [1]. Known cases of aplastic anemia after the history of acute viral hepatitis have been also recorded which is likely related to capacity of hepatitis virus to changing cells karyotype. Also Epstein-Barr virus and parvoviruses are likely to be possible causes of development of anemia disease. Among all types of illness its hereditary form can be also pointed out – so called Fanconi's anemia [4]. It is difficult to define the factors of disease onset in almost half of the patients and scientists tend to classify it as idiopathic aplastic anemia.

In accordance with contemporary idea which is based on electronic-microscopic, histological and biochemical methods for defining pathogenesis of aplastic anemia three principal mechanisms can be identified: direct affection of pluripotential stem cells; change of stem cell environment in particular which leads to its dysfunction [5, 6]. The main factor for pancytopenia on the cellular and kinetic levels is connected with significant decrease of pluripotential stem cells (PSCs) as well as count of more mature progenitor cells of erythro- myelo- and thrombocytopoesis. A quality defect of residual stem cells is also important, which is represented as lack of capacity to produce enough number of descendant cells.

Defect of PSCs is regarded as a primary abnormality which is remarkable and might be enhanced under effect of various etiology factors.

Primary nature of likely PSCs defect – a leading cause in pathogenesis of acquired aplastic anemia (AAA) based on detecting a sharp decrease of colony-forming properties in cells of bone marrow. Likely abnormality could be even noticeable within a period of clinical-hematology remission and after detecting morphology defects of hematopoiesis of cells. All such deviations can be an evidence of functional deficiency of PSCs. It has been proved that along with PSCs count lowering over more than 10% compared to the normal ranges there is a misbalance between process of cells differentiation and proliferation with likely predominance of cells differentiation as the main cause of decreased red BM colony-forming properties [7, 8].

Clinical pattern of hematopoiesis distress is constituted by anemia of different degree of severity and thrombocytopenia with marked hemorrhagic syndrome (petechia, ecchymoses, gingival bleeding etc.). Development of infectious complications caused by gram-negative flora can be a sequel of severe neutropenia (pneumonia, otitis, pus formation, bleeding or fever of undefined genesis). Causes of anemia in the patients can be referred to as both abnormalities of erythropoiesis and causative factors of hemorrhages. Such patients commonly experience bleeding, skin paleness, weakness; lower tolerance to physical exercising as well as tachycardia which is also remarkable [9, 10, 11].

Sometimes disease takes a fast progressive course and results in death during several weeks or months; however, in the other cases disease takes a chronic course with alternating periods of exacerbation and improvements. Infrequently patients report a complete recovery from this disease [12, 13].

Significant place in treatment of hematology diseases was referred to BM transplantation and such a practice dates back to 1970s [5, 6]. Either patient's own BM cells (prepared in advance for autotransplantation) or donated BM stem cells (allotransplantation) were administered for the patients with anemia [8, 13, 14].

Nevertheless, despite of modern protocols use for treatment of aplastic anemia patients, significant achievements in therapy have not been reached yet. One of the conducted studies emphasizes some therapy results among the elderly: patients aged over 60 years in particular, who demonstrated worse results after treatment with inclusion of immune suppressive therapy combined with transplantation of stem cells [7, 8, 9, 10]. Complex treatment by use of FSCs tends to be one of perspective therapy methods and poses great expectations at recovery of BM functions among the patients.

2. Material and Methods

Study for 50 patients with diagnosed AAA was performed in Cell Therapy Center EmCell. Diagnosis and evaluation of the disease was performed in conformity with Camitta criteria (1976p.) which define AAA as illness with cellular characteristics of BM at the level less than 30% and severe pancytopenia – which are regarded like in the least 2 of criteria for peripheral blood assessment:

1. absolute neutrophil count, ANC < 0.5 x 10^9 /L
2. platelets count, PLT < 20 × 10^9/L
3. reticulocytes count < 1%

Biopsy and aspiration of BM for the purpose of morphology and cytogenetic investigations were conducted prior to establishing the firm diagnosis of AAA. All patients were checked up for paroxysmal night-time hemoglobinuria with the help of flow cytometry.

Fetal stem cells (FSCs) were administered for all 50 patients who underwent complex treatment in the clinic. Patients received immune suppressive therapy before FSCs transplantation which included administration of antithymocyte globulin (ATG) in a dose of 20 mg./kg/day for 5 days intravenously along with adequate maintenance therapy: blood components, antibiotics, sterilization of intestines using broad spectra of antibiotics; treatment by antifungal and antiviral agents both in monotherapy and in combination with i.v. infusions of cyclosporine 3-5 mg./kg and prednisolone 2 mg./kg per day.

Response to treatment was identified as lacking criteria of aplastic anemia and assessment was made over 3 months after FSCs transplantation.

The main group of this study constituted 50 patients in age from 13 to 43 years (their average age made up 27±5 yrs.). Anemic syndrome has been identified in all patients under study with characteristic commonly anemic features as: weakness, lethargy, pallor of skin, tachycardia, and systolic murmur of different severity.

Infectious syndrome was diagnosed in 20 (40%) of the patients. Fever of unknown genesis remarkable for 10

patients (20%) was regarded as the most frequent sign of infectious syndrome. Pneumonia was recorded in 7 (14%) of patients, as well as generalized herpetic infection was characteristic only for 6 patients (12%). Usually, infectious complications in the patients were associated with gram-negative flora (please, refer to Tab.1).

Table 1. *Allocation of patients suffering from aplastic anemia according to the clinical syndromes.*

Clinical syndrome	Number of patients, n	Percentage of patients, %
Anemic	50	100
Infectious:	20	40
Fever of unknown genesis	10	20
Pneumonia	7	14
Generalized herpetic infection	6	12
Combination of anemic, hemorrhagic and intoxication syndromes	3	6

A combination of anemic, hemorrhagic and infectious syndromes at disease early onset has been just reported by 3 patients under study (6%) (Please, refer to Tab. 1).

Mild hepatomegaly was demonstrable in 4 children patients (8%) with severe form of AAA. Peripheral blood count results were remarkable for the expressed anemia in the patients. Ranges of hemoglobin revealed fluctuations starting from 60 g./L., whereas erythrocytes count ranged from 1.8 to 3.2 x 10^{12}/L. In severe forms of AAA (30%), anemia was less pronounced and hemoglobin levels were not lower than 80 g/L. Reticulocytes count constituted from 0.3 up to 2%. Majority of patients with AAA had normocytic anemia whereas 8 patients (16%) were diagnosed with macrocytic type of illness.

Level of platelets composed from 10 to 40 x 10^9/L and in this respect 6 patients had isolated thrombocytes in blood smears after we made all laboratory investigations.

6 patients underwent conservative treatment which included empiric antibiotics therapy and infusion of erythrocytes mass and/or platelet concentrate. We studied the patients who were administered FSCs whereas immune suppressive treatment during our investigation had not been performed for the patients.

Provisional values which were analyzed in all patients included: age, sex, leucocytes count, hemoglobin levels, as well as counts of neutrophils, platelets and reticulocytes. All statistic data identified were used for analysis of covariance.

Univariate and multiple variate analyses were performed for evaluation of all factors affecting the ranges of overall survival.

For a purpose of simplified representation of the results, all provisional values have been classified in accordance with Camitta criteria along with influence of covariance on survival of the patients.

Overall survival rates obtained by use of Kaplan-Meier estimator were also evaluated based on Logrank test.

Kaplan-Meier estimator

Method of nonparametric analysis was used for compilation of the tables describing rates of mortality and survival among the patients. This method combines the main calculated chances of survival and assessment which stipulates censorship by means of a sampling which is likely to be obtained in a random manner. Time intervals which are calculated always tend to be over as soon as adverse event happens in a patient (death, attrition etc.) and respectively those intervals are uneven.

Logrank test

This test is a non-parametric criterion used for comparison of 2 curves for survival among the patients. Null-hypothesis is a basic one to prove that survival rate in the groups is similar and all deviations are likely to be accidental. Application of logrank test is grounded on three main acknowledgements: two samplings compared are independent and changeable, whereas attrition in both samplings is the same and all functions of survival among the patients are associated.

Cox-regression analysis was also used for univariate and polivariate factors which affected the system of data processing. P score which is less than 0.05 was considered to be a statistically significant.

Regression analysis

Regression analysis is a chapter of mathematic statistics devoted to the methods of analysis of interrelation between one measurement and the other. As opposed to correlation analysis, this method does not deal with definition of how much significant particular interrelation is, but rather with search for the model of likely interconnection marked as a function of regression.

For treatment we used cryopreserved suspensions including pluripotent stem cells (PSCs) extracted from 7-12 weeks gestation human cadaveric fetuses which were received as a result of legal abortions owing to social indications or for the reason of family planning. All women signed informed consent for medical use of proper abortive material. Collection of material was made by following ethical-moral and legislative principles of work with biological tissues. All donors of the aborted material were practically healthy women and they revealed negative test results to hemic infections.

Biotechnology process of suspension preparing accounted for stem cells extraction from fetal liver; assessment of stem cells viability as well as their programmed cryopreservation; bacteriology and virology studies. Immediately before FSCs administration cryopreserved suspension was defrosted using a water-bath at temperature of 37°C along with additional assessment of stem cells viability.

Cryopreservation was made under protection of dimethyl sulfoxide 5% (DMSO) according to 3 stage program of freezing with initial speed of 1°C/min. and initiation of granulation. Stem cells viability was directly evaluated prior to administration along with trepan blue application as a staining method. Calculation was performed in parallel using Goryaev chamber and 1450001 TC10 TM Automated Cell Counter.

Prior to freezing stem cells viability in a suspension constituted 83.0 ± 3.0%. After storage at the low temperature cryobank (t -196ºC) and following suspension heating in a

water bath at temperature +37.5 ± 0.12°C stem cells viability rate was not less than 70.3 ± 1.02%.

The patients were administered fetal liver stem cells using i.v. drip-feed infusions after cells were washed off in DMSO. Volume of therapeutic dose for each administration was individually selected, but it constituted not less than 0.3 mL of cell suspension which included not less than $0.20x10^8$/mL nucleated cells and CD34+ progenitor cells ranging from 0.60 to $3.54x10^6$ /mL per each administration. Amount of viable stem cells in suspensions made up 70.0±10.0%.

All patients signed informed consent prior to beginning of treatment. General clinical examination was performed prior to treatment and over 1, 3 and 6 months after FSCs therapy.

Patients' state was evaluated with the help of general blood tests, absolute count of neutrophils, platelets and reticulocytes.

Detecting consistence of fetal hemoglobin was conducted with the help of Kleihauer–Betke test, where isolated stained red blood cells are usually demonstrable.

Kleihauer–Betke test

Method. Elution of hemoglobin using acid with additional staining of blood smears allows detecting erythrocytes which have hemoglobin F, because such cells preserve coloration as opposed to unstained erythrocytes which commonly include hemoglobin A.

3. Results

Over 8-12 hours after FSCs administration patients reported improved general condition, better appetite, less headache and reduction of weakness in their body. No allergic reactions were reported by the patients under study and their body temperature was not elevated.

For the 1st day after FSCs transplantation we recorded moderate increase of erythrocytes over 5-7%, whereas elevation of leucocytes count over 15-25% was noticeable on day 7 after therapy. As for the number of platelets, it was maintained unchanged during the first 7 days of FSCs therapy.

All results which revealed changes in laboratory values for the 1st day were not significant. After testing of peripheral blood particles of erythrocytes containing fetal hemoglobin were elevated up to 11% for day 10 after treatment using FSCs, if compared to day 3 where the same value was higher by 3%. After investigation of myelogram in patients a mild increasing of all lineages of hematopoiesis was characteristic for day 14. We observed further elevation of erythrocytes count including fetal hemoglobin over 45-50%. Significant increase of platelets count within peripheral blood was demonstrable during day 21 after FSCs treatment and made up 140-150%, and later the same tendency to thrombocytes count growth remained, to the extent of stabilization of cell parameters (see Tab. 2).

After the study patients were leading active mode of their living and stayed under hematologist's observation; immunosuppressive therapy was not conducted, hematology parameters remained within the normal limits.

Along with laboratory control we performed interviewing for the patients with subjective evaluation of their condition (see Figure 1). As it is outlined by the diagram, significant improvement of the patients' state is characteristic starting from day 7 when our patients reported improved general condition, appetite and sleep along with higher tolerance to physical exercises; right up to the day 14 better general well-being was recorded in over 90% of patients.

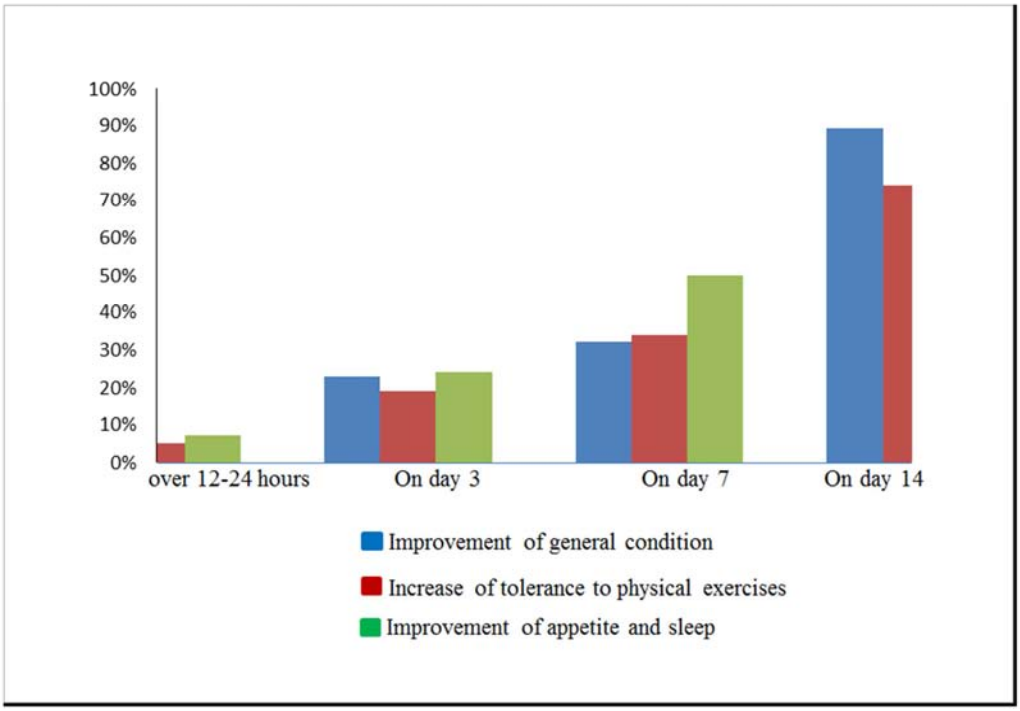

Figure 1. *Assessment of the subjective state of the patients.*

Table 2. *Average dynamics of laboratory values in patients after FSCs administration.*

Values:	over 7 days	over 14 days	over 21 days
erythrocytes × 10×12/L	1.9±0.20	2.2±0.18	4.02±0.4
hemoglobin g/ L	61±5.4	80±7.3	130±12
platelets × 10^9/ L	22±2.4	24±2.3	54±8.7
leukocytes × 10^9/ L	1.6±0.15	1.9±0.18	4.9±0.47
reticulocytes × 10^9/ L	Not identified	13±1.2	32±1.3

Notes:
*- $p<0.05$ – significance between values before and after FSCs treatment;
**- $p<0.001$ – significance between values before and after FSCs treatment;

As one can see from the graphical charts above we observed general positive dynamics which was recorded within the whole period of our study. Patients demonstrated regeneration of all lineages of hematopoiesis and platelets count elevation was significantly slower in comparison with the count of erythrocytes.

4. Discussion

Experimental use of human fetal material raises much disputes and controversies. Clinical use of human fetal tissues is not supported being even prohibited in some countries – among those are the USA and countries of European Union. In the other countries, where Ukraine belongs to, this biological material can be experimentally used as far as woman makes her decision on abortion and donating fetal tissues in accordance with all ethical, moral and legal principles. Taking into account real therapeutic benefits of fetal material with its enormous property to help many people with incurable diseases, it would be simply inhumane to leave out such an opportunity to save one's life or improve life quality of the others. Taking into account our not so huge, however, very optimistic experience with use of FSCs which preserve their capacity to transform into functional specialized cells of different body tissues, scientists lay great expectations on their effective use in clinical practice.

Along with this, there are fears of some experts which are related to probability of rather unfavorable side effects of stem cell therapy. There are data in literature in regard to inappropriate differentiation of cells where stem cells are believed may result in tumors – teratomas. During our study we used FSCs only, which have no oncogenic properties and no one of treated patients experienced similar processes. One more side effect after FSCs transplantation is a possibility of host reaction to transplanted stem cells; however, as long as HLA receptors are immature in the fetuses of 7-12 weeks gestation, rejection of fetal stem cells cannot take place. During a period of observation no single case was recorded by us in accordance with general clinical criteria. Therefore, one can conclude that traditional therapy with inclusion of FSCs is a perspective and safe method of treatment for aplastic anemia.

At this study we experienced significant advantages which were directed at pathogenesis of the disease and renewal of functions of hematopoiesis in regard to recovery of all lineages of blood formation. Almost twofold elevation of hemoglobin level was observed in our patients. Kleihauer–Betke test proved that such an increase was significant by virtue of fetal hemoglobin. One can conclude about functional full value of erythrocytes which was formed owing to FSCs and this contributes to estimation of overall disease course: improvement of general condition of the patients, change of coloration of mucous membranes from pallor to pink, improvement of appetite and increased tolerance to physical exercises among patients. In addition, significant effect of FSCs on megakaryocyte lineage of blood formation was established which could reflect itself as an elevation of platelets count over more than 2 times on day 21 after treatment and this value was likely statistically significant. Functional full value of thrombocytes was represented by reversal of signs of hemorrhagic syndrome, disappearance of petechial spots and mucosal bleeding after minor traumas etc. Simultaneously, with regard to platelets role for the processes of vascular wall metabolism one can summarize on improvement of microcirculation and condition of vascular blood flow. Before beginning of treatment 7 patients were diagnosed with pneumonia and they sustained FSCs therapy without particularities – disease course was without complications and respiratory failure in these patients did not exceed grades 0-1 of respiratory insufficiency. In respect to fever of unknown etiology – this problem was well-managed in all patients when the level of leukocytes was elevated up to $1.9\pm0.18\times10^9$/L, which was remarkable for day 7 and contributed to reduction in amount of prescribed antibiotics along with correlation of such dose regimens without substantial degrade in advantages of ongoing treatment. Special attention was concentrated on safety of this study and continuous monitoring for vital functions of the organism: arterial blood pressure, rate of respiration, level of blood oxygenation and body temperature control among patients. It has been established that FSCs do not cause pathological changes which might be life-threatening for the patient. Application of FSCs for treatment of patients with aplastic anemia can allow us simplifying the existing schemes of chemotherapy and reliable dose reduction looks promising – in the simplest terms; all this will affect better tolerance to treatment among the patients and their faster recovery after it. An attempt to achieve remission of aplastic anemia for a prolonged period of time after combined treatment with administration of FSCs can be identified as the principal purpose of this treatment. However, a range of questions still exists which demand the answer and all new clinical studies will eventually take precedence for this.

All results we acquired are much likely encouraging with regard to a complex treatment of the patients with aplastic anemia by use of standard therapy along with FSCs which might possibly substantiate FSCs use as a therapy of choice if transplantation of BM cannot be accessible for some patients. In particular, this refers to those patients who are included to

the waiting list for BM transplantation.

From this perspective it is too early to raise an idea about significance of FSCs for recovery of human BM cells; nevertheless, one may impose great expectations that major scientific studies in the nearest future will ultimately explicate the aspect of FSCs interaction with proper patient's stem cells as well as treatment potential in respect to protective influence on BM and patient's organism in particular.

5. Conclusions

Treatment by use of FSCs was performed at our clinic facility. Among all positive advantages after FSCs therapy use restoration of all lineages of hematopoiesis is the most significant that is an etiology factor for reversal of hemorrhagic, infectious and anemic syndromes. After complex therapy using stem cells we observed recovery of BM functions which was presented as restoration of thrombocytes and erythrocytes lineages of hematopoiesis as well as recovery of the level of leukocytes within a range of $4.9\pm0.47 \times 10^9$/L. As a result of FSCs treatment we reached improvement in hemoglobin levels and erythrocytes counts which were established by means of calculation of fetal hemoglobin. Administration of FSCs significantly increased life quality of our patients who presented improvement of their general condition and appetite as well as increased tolerance to physical exercises. With recovery of leukocytes count we observed stabilization of body temperature in the patients as well as reversal of fever of unknown genesis. Furthermore, the patients with pneumonia revealed a slight disease progress without development of complications.

Our study proved that FSCs administration is a safe method and no graft-versus-host reaction or any other adverse effect was recorded by the patients both at once after treatment using FSCs and within the long-term perspective.

References

[1] Guidelines for the Diagnosis and Management of Adult Aplastic Anaemia; British Committee for Standards in Haematology (2015).

[2] Guo D, Liu Q, Li B, et al; Severe aplastic anemia preceding acute monocytic leukemia in an adult with acquired trisomy 21: A case report. Oncol Lett. 2014 Feb; 7 (2): 565-567. Epub 2013 Dec 3.

[3] Biswajit H, Pratim PP, Kumar ST, et al; Aplastic anemia: a common hematological abnormality among peripheral pancytopenia. *N Am J Med Sci.* 2012 Sep; 4 (9): 384-8. doi: 10.4103/1947-2714.100980.

[4] Dror Y, Freedman MH. Inherited forms of bone marrow failure. In: Hoffman R, Benz EJ, Silberstein LE, Heslop HE, Weitz JI, Anastasi J, eds. Hematology: Basic Principles and Practice. 6th ed. Philadelphia, PA: Elsevier; 2013.

[5] Rovó A, Tichelli A, Dufour C, SAA-WP EBMT. Diagnosis of acquired aplastic anemia. Bone Marrow Transplant 2013; 48: 162.

[6] Bagby GC. Aplastic anemia and related bone marrow failure states. In: Goldman L, Schafer AI, eds. Goldman's Cecil Medicine. 25th ed. Philadelphia, PA: Elsevier Saunders; 2016.

[7] Tichelli A, Marsh JC; Treatment of aplastic anaemia in elderly patients aged >60 years. *Bone Marrow Transplant.* 2013 Feb; 48 (2): 180-2. doi: 10.1038/bmt.2012.224. Epub 2012 Nov 26.

[8] Peinemann F, Bartel C, Grouven U. First-line allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared with first-line ciclosporin and/or antithymocyte or antilymphocyte globulin for acquired severe aplastic anemia. Cochrane Database Syst Rev 2013; CD006407.

[9] Dasouki MJ, Rafi SK, Olm-Shipman AJ, et al. Exome sequencing reveals a thrombopoietin ligand mutation in a Micronesian family with autosomal recessive aplastic anemia. Blood 2013; 122: 3440.

[10] Kordasti S, Marsh J, Al-Khan S, et al. Functional characterization of CD4+ T cells in aplastic anemia. Blood 2012; 119: 2033.

[11] Li Y, Li X, Ge M, Shi J, Qian L, Zheng Y, et al. (2011) Long-term follow-up of clonal evolutions in 802 aplastic anemia patients: a single-center experience. *Ann Hematol* 90: 529-537. pmid: 21207031.

[12] Scheinberg P, Young NS (2012) How I treat acquired aplastic anemia. Blood 120: 1185-1196. doi: pmid: 22517900.

[13] Eapen M, Le Rademacher J, Antin JH, et al. Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia. *Blood.* 2011.

[14] Valdez JM, Scheinberg P, Nunez O, et al. Decreased infection-related mortality and improved survival in severe aplastic anemia in the past two decades. Clin Infect Dis 2011; 52: 726.