Medical Sciences

Using of Blood from Cadaveric Donor in Orthotopic Liver Transplantation

Mogely Khubutia[*], Sergey Zhuravel[**], Vladimir Guliaev[**], Valentina Nikulina[**], Murad Novruzbekov[**], Svetlana Kabanova[**], Valeriy Khvatov[**]

* *Member of the Russian Academy of Sciences, N.V. Sklifosovsky Research Institute for Emergency Medicine. Moscow, Russia*
**Healthcare Department of Public Healthcare Institution, N.V. Sklifosovsky Research Institute for Emergency Medicine, Moscow, Russia*

ABSTRACT. The purpose of the article is to represent the experience of usage of allogenic red blood cells received from cadaveric donors in liver transplantation. The study included 60 recipients who underwent orthotopic liver transplant procedures. One group of 30 intraoperative recipients were administered red blood cells received from the donor liver. In group 2 (n = 30) blood of cadaveric donor liver was not used. Autologous red blood cells (ARBC) were harvested during the operation of the removal of organs from the brain of the dead donor (BDD). This application of ARBC resulted in the decline of the use of allogenic donor's blood. In addition, ARBC provided an immunosuppressive effect and prophylaxis rejection in an early postoperative period. © *2014 Bull. Georg. Natl. Acad. Sci.*

Key words: *liver transplantation, blood transfusion, cadaveric donor*

Orthotopic liver transplantation (OLT) is the treatment of choice for patients with acute or chronic end-stage liver disease, unrespectable primary liver tumor, and metabolic disorders. Historically, OLT has been associated with considerable blood loss and the need for transfusions.

Pre-anhepatic phase is associated with blood loss due to dissection of the liver. Haemodilution and hypothermia, resulting in altered coagulation status, add on to the pre-existing anaemia and coagulopathy. Aggressive correction of the coagulation status may lead to hypervolaemia and increasing blood loss in addition to the effect on ionized hypocalcaemia. During the anhepatic phase there is ionized hypocalcaemia, failure of clearance of thromboplastin and a rapid rise in tissue-type plasminogen activator with increase in plasmin activity and a hyperfibrinolytic state in addition to the pre-existing coagulopathy[1].

The rationale of acute normovolaemic haemodilution is to reduce the Htc before the intraoperative bleeding, in order to limit the loss of red blood cells [2]. The efficacy of acute normovolaemic haemodilution in reducing the need for allogeneic blood transfusion, however, does remain doubtful.

Table 1. Characteristics of patients

	1group	2group	p
Age	45.5±5	45±9	NS
Gender			
Male	18	17	NS
Female	12	13	NS
Meld	20±7	18±8	NS

Intraoperative blood salvage and retransfusion of the autologous blood is a cost-effective method to reduce the need for allogenic red blood cells in OLT provided there are no contraindications to cell savage and the centre is a high turnover (with a minimum of 80 cases of cell savage per year) [3].

In spite of many improvements that have reduced the blood component requirements, substantial numbers of transfusions are still needed in liver transplantation.

Despite the risks associated with transfusion, the medical community continues to view blood as a safe and abundant product.

Intra-operative transfusion support is aimed at correcting acute anemia and treating clotting disorders and secondary forms of thrombocytopenia. In fact, a massive, acute hemorrhage can cause hypovolemic shock with consequent tissue hypoxia, acidosis, hypothermia and systemic inflammatory response, which can trigger disseminated intravascular coagulation (DIC) [4-10].

In this article, we provide an effective strategy to accomplish orthotopic liver transplantation with transfusion .

Aim

The objective of the present study was to analyze the intraoperative usage of allogenic red blood cells received from cadaveric donors , as well as to determine their impact on immunological parameters in the postoperative period in liver transplant recipients

Materials and Methods

The study included 60 recipients who underwent orthotopic liver transplant procedures.

One group of 30 intraoperative recipients were administered red blood cells received from the donor liver. In group 2 (n = 30) blood of cadaveric donor was not used.

Overall, general patient characteristics did not significantly differ between the two groups (Table 1, 2).

Technique to Obtain Red Blood Cells

Autologous red blood cells was performed during surgery harvesting of organs from brain death donors (BDD).

After mobilization of the liver was cannulated inferior vena cava below the renal veins, was applied clamp on the aorta and within 30-40 seconds the blood from the inferior vena cava was aspirated the autotransfusion device. The donor's blood passes through the system with citrate and comes into centrifuge.

Blood sampling was stopped when blood pressure (sis) decreased <90mm Hg. Thereafter, the aortic clamp removed, the inferior vena cava was ligated above the cannulation and began flushing solution "Custadiol" through the aorta. At the same time continued to aspirate blood from the donor inferior vena cava, which comes from the lower extremities (ligation of the inferior vena cava above the cannulation of solution is precluded from entering into the autotransfusion device. Red cell concentrate (Ht = 70-80%) was obtained by the mode High quality wash.

The cellular component of autologous red blood cell mass analyzer FACS Calibur, the BD Multitest IMK kit (Becton Dickinson, USA) was investigated. Before the appointment of the recipient red blood cells combined by the usual method.

Washed red cell concentrate was used in liver transplant recipient after venous liver reperfusion.

Immunosupression

Immunosuppression included calcineurin inhibitor, mycophenolate mofetil, steroids and monoclonal antibodies to an anti-interleukin 2 receptor.

Table 2. Indications for liver transplantation

	1 group n (%)	2 group n (%)
HCV cirrhosis	12 (40)	11 (37)
HBV cirrhosis	8 (27)	9 (30)
HCV + HCC	5 (17)	3 (10)
Primary biliary cirrhosis	2 (7)	4 (14)
Alcoholic cirrhosis	2 (6)	1 (3)
Wilson disease	0	1 (3)
Primary sclerosis cholangitis	1(3)	1 (3)

Episodes of acute cellular rejection diagnosed histologically (rejection activity index Banff). Liver biopsy was performed in case of registration of the increase of liver enzymes in the postoperative period. Treatment of acute rejection crisis included prescription solumedrol 500 mg for 3 days.

Immunologic study included determination of blood, key populations and subpopulations of lymphocytes by flow cytometry. Content was quantitated CD3+, CD4+, CD8+, CD19+, NK-cells by flow cytometry for 2, 5, 7, 12,14 days after transplantation. A comparative analysis of the main content of lymphocyte populations in the peripheral blood of patients in patients who received perioperative period AEM - group 1 and -2 have not received HEA group.

Statistical analysis

Descriptive statistics are expressed as number (proportion) or mean±SD or mediana (25%₀, 75%₀) in the case of abnormal distribution. Differences between groups were examined using Mann-Whitney. Data were examined using Statistica 8. P<0.05 was considered significant.

Results

The whole blood volume drawn from the donor was 1.300 ± 350 mL.

The average count of infused autologous red blood cells (ARBC) in the 1 group was 445±183cells/ïl.

Investigation of the autoblood cellular component, which is a plasmaless transfusion medium in the form of cellular concentrate in isotonic saline solution. The spectrotype in this transfusion medium: red blood count is in average 7.8×10^{12} cells/L, white blood count in average 7.5×10^{9} cells/L, platelet count in average 57×10^{9} cells/L.

As seen from the Table, ARBC transfusion resulted in credible increase of systemic vascular resistance, pulmonary capillary-wedge pressure, central venous pressure, Hb and oxygen delivery. The results obtained show that ARBC has a volume filling effect in replacement of blood loss, and in combination with infusion therapy it ensures improved functional condition of internal organs and overall prognosis for the patient.

BP- blood pressure, CVP - central venous pressure, CI – cardiac index, PCWP - pulmonary capillary wedge pressure, SVR - systemic vascular resistance.

Table 3. Comparison cells component whole blood and red blood cells

	Sample venous blood cadaveric donor	Sample ARBC
Hematocrit об %	23±3	69±3*
Erythrocytes x 10^{12} cell/µl	2.8±0.9	7.8±0.7*
White blood cells x 10^{9} cell/µl	8.5±0.3	7.5±0.4
Platelets x 10^{9} cell/µl	83±15	57±7*
Hemoglobin g/L	75±4	203±9*
Free hemoglobin g/L	5.0±0.5	0.20±0.09*

ARBC- autologous red blood cells
*-P<0.05

Table 4. Change of hemodynamics, oxygen delivery and Hb after transfusion

Parameters	Before transfusion	After transfusion	Normal range
BPmean, mm Hg	70±12	75±11	82–102
CVP, mm Hg.	6±2	9±2*	1–9
PCWP mm Hg	8±1	12±2*	0–12
CI l/min/m^2	3,1±0,6	3,2±0,5	2,8–3,6
Oxygen delivery, ml/min/m^2	640±90	988±110*	520–720
SVR dynes·sec·cm^5	620±85	800±120*	1200–2500
Hв g/l	67±7	88±6*	120-160

* - P < 0,05

It is obvious that improved oxygen delivery was related to increased Hb level. It is known that oxygen delivery to tissues is mainly defined by cardiac output and oxygen content in arterial blood. O_2 delivery is calculated as a product of O_2 production and cardiac output

$$DO_2 = CaO_2 \times CO.$$

O_2 content is defined as the total oxygen transferred by Hb plus oxygen dissolved in plasma.

$$CaO_2 = (Hb \times 1.34 \times SaO_2) + (0.0031 \times PaO_2),$$

where 1.34 - O_2 amount (ml) in 1g Ha, and 0.0031 – O_2 solubility in plasma (mL/dL)

Consequently, the Hb major function - capability to be reversely bound with oxygen – has been preserved in ARBC, and after its application, maintaining and restoration of oxygen transportation function of blood was registered.

We found no complications when using ARBC.

Thus, ARBC obtained from a cadaveric donor preserves therapeutic efficiency; transfused red cells are viable and perform their functions.

Investigation of the demand for allogeneic packed red blood cells in liver transplantation showed that transfusion of autologous red blood cells allowed abandoning donor's packed red cells in 73% of cases.

In the control group, the number of such patients was 33% with compared blood loss.

The results of our investigation showed various number of acute rejection episodes in compared groups. Thus, in the ARBC group, a rejection episode was developed in one patient (3%) versus 26% (4 patients) in the control group.

Changes in the quantitative composition of lymphocytic populations of periphery blood in patients from groups under investigation during the first 2 weeks after OLT.

In patient from the experimental group, larger number of CD3+ white cells was registered, as compared to the control group patients during the investigation period specified. The most significant differences in the number of CD3+ white cells were registered on the 14-th day. Exceeding the number CD3+ white cells in the experimental group on the 14-th day was 38.9% as compared to the control group, but differences are not statistically credible (Table 6).

Data of statistical analysis of lymphocytic populations in patients from both groups on the 14-th day after OLT.

Average count of CD4+ lymphocytes in the experimental group patients on the 2nd day after LT ex-

Table 5. Blood loss demand for allogeneic packed red blood cells in liver transplantation

	1 group (n=30)	2 group (n=30)	P
Blood loss	2600 (700, 5000)	2800 (500, 8500)	>0.05
Use allogenic packed red blood	33%	73 %	<0.05

Table 6. Quantity lymphocyte populations on day 14 after liver transplantation

	1 group		2 group		p	P-value
	Mean	Median; 25 and 75%$_0$	Mean	Median; 25 and 75%$_0$		
CD3+ cell/ 1	681.7	491.0; 189.0; 901.0	490.9	514; 92.0; 688.0	-	0.5081
CD4+ cell/ 1	106.3	79.0 27.8; 133.3	111.0	92.0 33.5; 161.5	-	0.7546
CD8 +cell/ 1	187.1	163.5 55.5; 263.0	213.7	208.0 73.0; 296.0	-	0.7241
CD16+CD56+ cell/ 1	80.5	58.0 34.25 ; 104.0	63.7	64.0: 22.0; 95.0	-	0.5757
CD19 +cell/ 1	354.8	325.0; 159.8; 392.0	171.3	118.0; 38.0; 364.0	<0.05	0.0401

ceeded the count of cells in the control group patients by 12.8%. In the following day, CD4+ lymphocyte count was decreased in the experimental group patients as compared to the average value in the control group. There was registered no credible difference in CD4+ lymphocyte count between the groups by the 14-th day.

The count of CD8+ effector lymphocytes in the experimental group patients was registered to be 0.6-52% less than in the control group patients during the observation period. On the 3rd day after LT, the number of CD8+ lymphocytes in the experimental group was registered to be 52.1% less than in the control group, but differences are statistically unreliable. By the 14-th day, the number of CD8+ lymphocytes in the control group was registered to be 21.6% less than in the control group, and differences are unreliable. The comparative analysis of NK-cell count in patient of both groups showed that the NK-cell count in the control group patients exceeded, but statistically credible difference were not found.

The analysis of B– lymphocyte (CD19+) count in comparison groups showed most significant differences in B– lymphocyte content in patients of the

groups under analysis. B–lymphocyte count in the experimental group patients exceeded that of the control group by 1.3 – 3.1 times. Statistically credible differences were registered on the 5th and 14th day after LT (p<0.05) (Fig.1).

Discussion

At this stage the basic direction of immunosuppressive strategies during transplantation of organs to maintain a transplant function is exhaustion of effector clonals of lymphocytes that are CD8 + and CD4+ lymphocytes at the early stage [17,19].

The best solution to the problem of maintenance of a transplant function is creation of tolerance to the allogenic organ in absence of minimum immunosuppressive therapy or when this therapy remains the same.

Tolerance implies amionectic immune reaction or complete unresponsiveness (anergy) of the immune system of recipient for alloantigens donor [11, 14, 19].

The first step to tolerance is creation of chimerism, i.e. coexistence of the immunesystems of the donor and the recipient [13, 14, 23, 24].

Chimerism can be induced, increasing the presence of allogenic cells of the donor by infusion
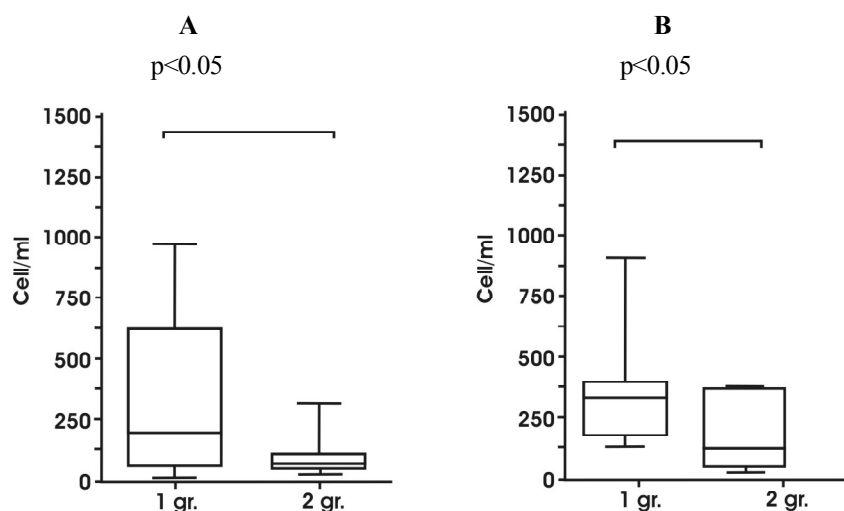
Fig. 1. B– lymphocyte count (CD19+) on the 5th (A) and 14th days (B) after transplantation.

of marrow or leucocytes of donor while transplantating. Thus, chimerism (microchimerism) and tolerance imply protracted persistence of immunocompetency cells of the donor in the organism of the recipient and absence of expressed proliferation of effector clonals of cells sensibilized to the alloantigen [13, 17, 18].

In our research to strengthen an allogenic effect on the organism of the recipient, we used ABCD containing the leucocytes of the donor organ.

The change of amount of immunocompetency cells defined by us could be conditioned by including the central mechanisms of immunoregulation of the recipient, as it is known that the donor's DC are diagnosed in the recipient's thymus already on the fifth day after infusion of the donor's leucocytes [17].

Undoubtedly, the presence of allogenic cells in the of recipient's organism creates the environment for correlation of the donor's DC and the recipient's thymocytes, that can change the processes of alloreactive clonals forming and assist central chimerism forming .

As a result, proliferation of allogenic clonals of lymphocytes can go down, what,apparently, takes place since the 5th day and for subsequent days after OLT.

Similarly, it is impossible to eliminate influence of T-regulatory (Treg.) cells on the processes of forming of peripheral tolerance after transplantation. At this cell of study of mechanisms of tolerance it isconsidered that Treg possessing suppressant properties play a significant role in forming a tolerogenic effect [12, 15, 25, 26].

Decline of CD8+ lymphocytes since the first twenty-four hours after OLT, probably, is related to influence of Treg.cell on immunopathogenetic processes taking place in peripheral lymphonoduss, as it is known that CD8+ lymphocytes are one of main targets for the regulator (suppressant) action of Treg. As a result of this influence, proliferation goes down and the embryonization of CD8+ lymphocytes is repressed, the secretion of cytokines goes down, thus the suppressor effect of Treg shows up in the first twenty-four hours after activating [12].

The reliable increase of number of Â- lymphocytes is marked in the first 2 weeks, and according to some authors' research, there is an increase of number of B- lymphocytes in subsequent months after transplantation.

Some further research is required [27] to realize the value of megascopic number of B- lymphocytes on survivability of the transplant.

Thus, the results of our research testify that the mass of red corpuscles of liver as a donor can effectively be used for correction of bloodless. Application of ARBC durin the intraoperative period was an

effective method of indemnification of bloodless. This application of ARBC resulted in the decline of the use of allogenic donor's blood.

In addition, ARBC provided an immunosuppressive effect and prophylaxis rejection in an early postoperative period.

საზედიცინო მეცნიერებანი

გვამური დონორის სისხლის გამოყენება ღვიძლის ორთოტოპიული ტრანსპლანტაციისას

მ. ხუბუტია*, ს. ჟურაველი**, ვ. გულიაევი**, ვ. ნიკულინა**,
მ. ნოვრუზბეკოვი**,ს. კაბანოვა**, ვ. ხვატოვი**,

* *რუსეთის მეცნიერებათა აკადემიის წევრი, მოსკოვი, რუსეთი*
***ნ.ვ. სკლიფოსოვსკის სახელობის სასწრაფო საზედიცინო დახმარების კვლევითი ინსტიტუტი, მოსკოვი, რუსეთი*

ნაშრომში აღწერილია გვამური დონორებიდან აღებული ალოგენური სისხლის წითელი უჯრედების გამოყენების გამოცდილება ღვიძლის ორთოტოპიული გადანერგვისას. გამოკვლევა მოიცავდა 60 რეციპიენტს, რომლებმაც ღვიძლის ორთოტოპიული გადანერგვის პროცედურები გაიარეს. ერთ ჯგუფში შედიოდა 30 რეციპიენტი, რომლებთანაც ოპერაციის დროს დონორი ღვიძლის სისხლის წითელი უჯრედები გამოვიყენეთ. მეორე ჯგუფში (N = 30) გვამური დონორი ღვიძლის სისხლი არ გამოვგიყენებია. ოპერაციის დროს ორგანოების ამოღებისას გარდაცვლილი დონორის სისხლის ძარღვებიდან ავიღეთ ავტოლოგიური სისხლის წითელი უჯრედები. ამასთან ერთად, ავტოლოგიური სისხლის წითელმა უჯრედებმა უზრუნველყო იმუნოსუპრესიული ეფექტი და პროფილაქტიკა პოსტოპერაციული პერიოდის ადრეულ სტადიაზე.

REFERENSES

1. *MJ. Hannaman, ZG. Hevesi* (2011) Transplant Rev (Orlando) 25:36–43.
2. *TG. Monk* (2005) Anesthesiology Clin N Am. 23:271–81.
3. *SD. Phillips, D. Maguire, R. Deshpande, P. Muiesan, MJ. Bowles, M. Rela, et al*. (2006) Transplantation. 81:536–40.
4. Practice Guidelines for Perioperative Blood Transfusion and Adjuvant Therapies in: Anesthesiology (2006) 105:198–208.
5. *DIC E. Searle, A. Pavord, Z. Alfirevic* (2008) Best Pract Res Clin Obstet Gynaecol. 22:1075–88.
6. *IT. Pereboom, MT. de Boer, EB. Haagsma, HG. Hendriks, T. Lisman, RJ. Porte* (2009) Anesth Analg.108:1083–91.
7. *MT. de Boer, MC. Christensen, M. Asmussen, CS. van der Hilst, HG. Hendriks, MJ. Slooff, et al*. (2008) Anesth Analg 106:32–44.
8. *B. Nardo, R. Bertelli, R. Montalti, P. Beltempo, L. Puviani, V. Pacile, et al*. (2005) Transplant Proc 37:4389–92.
9. *IT. Pereboom, T. Lisman, RJ. Porte* (2008) Liver Transpl 14:923–31.
10. *W.N. Erber* (2002) Transfus. Apheresis. Sci. 27(1): 83-92
11. *A.A. Yarilin* (2010) Immunologia. M.: Medicine, - 745p.
12. *A.A. Yarilin* (2010) Immunologia. 3: 153-162.
13. *T.E. Starzl* (1998) Transplantacion Jul. 27. 66(2).
14. *T.E. Starzl, N. Murase, A. Demetris, M. Trucco and D. Fung* (2000) Seminars in Liver Diasease – **20**, 4.
15. *T. Patrick, Walsh, K. Devon, Taylor and A. Laurence Turka* (2004) J. Clin. Invest. 114:1398-1403, doi:10.1172/JCI200423238.
16. *J. Lerut* (2006) American Journal of Transplantation 6 (8): 1774–1780.
17. *M. Noris, D. Cugini, F. Casiraghi, N. Azzollini, LDe et al*. (2001) Journal of the American Society of Nephrology. **12,** 12.
18. *E. Thomas Starzl*. (2004) Chimerism and tolerance in transplantation, Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh. by The National Academy of Sciences of the USA.
19. *D. Golshayon, L. Buhler, R.I. Lechler, M. Pascual* (2006) Transplant International ISSN. Switzerland.
20. *A. Kubitskiy, J. Li, M. Lanzetta* (2007) Transplantation. **83**, 9: 1273-77.
21. *Demitris Starzl, N. Murase* (1992) Lancet 339:1579.
22. *CA. Huag, Y. Fusimoto Schier-DolbergR, VC. Murphy, et al*. (2000) J, Clin. Invest 105: 173-181.
23. *T.E. Starzl, N. Murase, A. Demetris, M.Trucco, AW. Thomas* (1996) Immunol. Today 17: 577-584.
24. *JM. Mathew, R. Gasia-Morales, A. Rosen, G. Ciancio, et.al* (2000) Transplantation 70: 1675-1682,
25. *L. Allison Bayer, Yu Aixin and R. Thomas Malek.* (2007) The Journal of Immunology, 178: 4062-4071.
26. *W. Li, K. Carper, XX, Zheng, CS. Kuhr, JD. Reyes, Y. Liang, DL. Perkins* (2006) Transplant Proc. Dec; 38(10):3205-6.
27. *Y. Peng, M. Ke, L. Xu* (2013) Transplantation. Jan 15;95(1):161-8. doi: 10.1097/TP.