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EFFECT OF TRANSPLANTATION OF CORD BLOOD TOTAL NUCLEATED CELLS ON THE MANIFESTATION AND PROGNOSIS OF REFRACTORY CONGESTIVE HEART FAILURE

ABSTRACT

The results of a prospective non-randomized observation study without control group to assess the course of heart failure in patients who underwent of cord blood total nucleated cells (CB TNCs) transplantation combined with traditional drug therapy have been presented.

MATERIALS. The study included 20 patients with congestive heart failure (CHF) IIA-IIB stage, functional class III-IV by the NYHA. CB TNCs transplantation was performed by a single intravenous dose of cell product «Cryopreserved human cord blood». Before and 1, 3, 6 and 9 months after CB TNC transplantation patients underwent echocardiographic study, the results of tests with the 6-minute walk determined exercise tolerance. The concentration of brain natriuretic peptide precursor (NT-proBNP) in blood was determined. The cardiovascular death risk was calculated using the scale MAGGIC.

RESULTS. Initial patients' status was characterized by the presence of severe heart failure with reduced contractility of the myocardium and increased risk of 1- and 3-year death. Traditional conservative therapy (beta blockers, ACE inhibitors, diuretics) was not effective. After CB TNC transplantation there was registered a significant improvement of general condition of patients, an increase in exercise tolerance and, therefore, reduce of HF functional class by NYHA (before transplantation average FC was 3.2, in the post-transplant period – from 2.1 to 2.8). Also after TNCs transplantation levels of biochemical markers of HF significantly decreased (before CB TNCs transplantation the level of NT-proBNP was 2370.3 ± 448.9 pg/mL, after CB TNCs transplantation – from 1198.6 ± 396.3 to 2300.7 ± 403.0 pg/mL) and the same was estimated death risk from HF (1-year – 10.1-37.4 %, 3 years - 9.1-42.3 % relative to the data of the initial state). Reduced HF manifestations after CB TNCs transplantation allowed to reduce significantly the diuretics dose.

CONCLUSION. Thus, transplantation of cord blood total nucleated cells in complex treatment of congestive heart failure has led to a greater efficiency of therapy and a significant reduce of CHF manifestations in patients.

KEYWORDS: heart failure, nuclei-containing cord blood cells, cell transplantation

Cardiovascular disease is the leading causes of morbidity, hospitalization and mortality in the world. Most of these diseases lead to heart failure (HF). Congestive HF is the most common cause of invalidization and mortality in the industrialized world [4]. The prevalence of congestive HF is estimated as an average of 1-2 % and significantly increases with the age [1, 8]. Congestive HF is the leading cause of hospitalization among the elderly population [3]. Thus, HF is a progressive medical, social and economic problem in the public health service. Current treatments usually only eliminate the symptoms and retard the progression of HF, but do not solve this problem completely [11].

The only radical and most effective method of treatment for end-stage HF today is heart transplantation [12]. The number of patients with

end-stage heart failure is increased constantly, but a number of heart transplantations has remained relatively stable in the world during the last decade [6]. Considering the above, constant search for alternative methods that would extend the life of patients with end-stage HF and improve its quality is being conducted [2, 11].

One of these alternative treatments for HF is cell therapy with different types of stem cells (SCs). Many of studies have shown promising use of little-studied source of SCs – cord blood – in patients with heart failure and reduced myocardial contractility [9].

THE PURPOSE of this study was to analyze the safety and effectiveness of transplantation of cord blood total nucleated cells in patients with refractory heart failure. To achieve this purpose the following **OBJECTIVES**

were selected: 1) to analyze the changes of heart failure manifestations and exercise tolerance in patients after transplantation of cord blood cells; 2) to analyze the short-term and medium-term effectiveness of conservative treatment after transplantation of cord blood cells; 3) to analyze changes in biochemical markers of heart failure and estimated cardiovascular disease mortality risk after transplantation of cord blood cells.

MATERIALS AND METHODS

The trial is based on the results of a prospective non-randomized observation study without a control group in 20 patients (clinical characteristics of patients are presented in Table. 1) that underwent cord blood total nucleated cells (CB TNCs) transplantation, combined with traditional drug therapy. The study was carried out under the R&D project of Shalimov National Institute of Surgery and Transplantology NAMS of Ukraine. The collection and storage of umbilical cord blood was performed in accordance with the current legislation of Ukraine, in compliance with generally accepted ethical aspects and the permission of the Coordination Center of Transplantation of Organs, Tissues and Cells of the Ministry of Health of Ukraine and approval of the medical ethics committee of Shalimov National Institute of Surgery and Transplantology on 29/07/2013.

The criteria for inclusion in the study group were age 20 to 60 years, HF stage IIA-IIB, functional class (FC) III or IV according to New York Heart Association (NYHA), reduced left ventricular ejection fraction (less than 35 %). At the start of the study the written informed consent was obtained. All patients undertake to comply with medication therapy and notify about all other drugs. Exclusion criteria of participants in the study were: patient's refusal to participate; severe complications; decompensated concomitant diseases or acute illness that can significantly affect the results of the study; the use of not recommended drugs; oncological or psychiatric anamnestic history (Tab. 1).

Collecting of cord blood and transplantation of the cell was carried out by the written informed consent of donor and recipient representatives. Cell viability and required biosafety parameters have been certified in the analytical passport that was provided for each individual dose of the cell drug by the manufacturer (Institute of Cell Therapy, Kyiv). For each dose of the cell drug there was performed a microbiological study and polymerase chain reaction to exclude markers of transmissible infections in cord blood and maternal blood (HIV 1/2, Treponema pallidum, Toxoplasma gondii, CMV, HCV, HBcorAg, HBsAg).

CB TNCs transplantation was performed as a single injection of washed from cryoprotector cell preparation «Cryopreserved human cord blood» washed from cryoprotector by intravenous infusion at a speed of 20–25 drops per minute in 200 mL of saline. The dose of cell preparation (4.5 mL suspension) included from $0.89 \cdot 10^9$ to $0.95 \cdot 10^9$ nucleated cells including mononuclear cells from $0.486 \cdot 10^9$ to $0.52 \cdot 10^9$ with viability 98–99 %.

Complex examination of patients was carried out before CB TNCs transplantation and 1, 3, 6 and 9 months after it. Echocardiographic examination of patients was performed by ultrasound scanner Aplio 500 (Toshiba, Japan). For the assessment of intracardiac hemodynamic parameters and systolic and diastolic functions of the cardiac muscle, conventional methods were used [5]. Clinical assessment of systolic and

Table 1. General characteristics of patients included in the study.

SEX, N (%)	Men	20 (100 %)
	Women	0
AGE, YEARS	Average value	49.6 ± 8.0
	Range	39–63
HF STAGE, N (%)	IIA	16 (80 %)
	IIB	4 (20 %)
STAGE AHA, N (%)	Refractory (D)	20 (100 %)
	III	16 (80 %)
FC NYHA, N (%)	IV	4 (20 %)
	Average value	24.8 ± 4.1
LVEF, %	Range	16–33

Note: NYHA – New York Heart Association; AHA – American Heart Association; LVEF – left ventricular ejection fraction

diastolic dysfunction by echocardiography data-tests were performed according to the «Guidelines for diagnosis and treatment of chronic heart failure». The results of echocardiography study after CB TNCs transplantation generalized by observations stages are presented in Table 2.

Exercise tolerance and functional class of HF was determined by the results of six-minute walk test (6MWT). Objectification of HF severity was performed by levels of the brain natriuretic peptide precursor (NT-proBNP) according to current recommendations [7]. The assessment of cardiovascular disease mortality risk was carried out using the Meta-analysis Global Group in Chronic Heart Failure (MAGGIC) score [10, 13].

Statistical analysis of the data was performed by conventional methods using two-sample Student's t-test with unequal variances. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Over the entire period of observation there was not registered an increase of clinical manifestations of HF, except two patients: one patient had HF decompensation caused by systematic abuse of prescribed drug therapy. Another patient had significant concomitant non-cardiac disease, which in combination with HF progressing led to the death of the patient on the 2nd month of observation.

CB TNCs transplantation allowed to improve significantly the general well-being of patients with HF refractory to conventional therapy (Tab. 3).

According to the table, there was registered a steady improvement of HF FC by NYHA till the sixth month of observation after CB TNCs transplantation. At the end of the 9th month of observation the average FC of patients slightly regress relatively to previous maximum values, but was significantly higher than the initial value.

The above changes of HF FC were confirmed by an increase in the distance covered during the 6MWT (Tab. 4).

VALUE	BEFORE TRANS- PLANTATION, N=20	AFTER 1 MONTH, N=18	AFTER 3 MONTHS, N=18	AFTER 6 MONTHS, N=14	AFTER 9 MONTHS, N=14
EF (%)	24.8 ± 4.1	25.3 ± 4.5	31.6 ± 4.8 *	33.3 ± 4.5 *	32.7 ± 4.9 *
ESI (mL/m ²)	79.7 ± 12.7	78.5 ± 16.1	71.1 ± 17.1	66.8 ± 8.4 *	67.0 ± 7.9 *
EDI (mL/m ²)	105.9 ± 14.7	104.3 ± 17.6	103.2 ± 19.0	100.4 ± 11.1	100.3 ± 11.7
SI (mL/m ²)	26.2 ± 5.2	25.9 ± 5.2	32.1 ± 5.9 *	33.5 ± 6.3 *	33.3 ± 7.5 *

Table 2. Systolic and diastolic left ventricular function before and after CB TNCs transplantation (M ± m).

Notes: EF – ejection fraction;
ESI – end-systolic volume index;
EDI – end-diastolic volume index;
SI – stroke volume index;
* – $p < 0.05$ compared with the initial state.

According to the table, the exercise tolerance increased significantly to the third month of observation after CB TNCs transplantation. In the period from the 3rd to the 9th month of observation, despite the negative trend relatively to the level of the best indexes, covered distance was significantly higher than the rates in these patients before CB TNCs transplantation ($p < 0.05$). Full compensation of HF clinical symptoms was achieved by the end of the 3rd month of observations, and a positive effect was kept to the end of the observation period.

Objective confirmation of the reduction of HF clinical manifestations was positive dynamics of the brain natriuretic peptide precursor. The most significant decrease in NT-proBNP level was registered in the 1st month of observation with the lowest level of it in the 3rd month. At the end of the 6th and 9th months of observation a tendency to decrease in NT-proBNP levels was also registered, as compared to the initial state.

During the observation by the end of the first month after CB TNCs transplantation there was a significant reduces of edema symptoms compared with the initial state. At reducing of HF manifestations an enhancement of diuretic efficiency was observed. After CB TNCs transplantation the average doses of diuretic drugs were reduced: 38,4-41,4 % for spironolactone (*Veroshpiron*, Gedeon Richter, Hungary) and 31,2-48,4 % for furosemide (*Borshchahivskiy CPP*, Ukraine), compared with the initial state. One patient after CB TNCs transplantation needed a 2-3 multiple intravenous injection of Furosemide (as a result of an abuse of prescribed drug therapy) followed by peroral administration of veroshpiron and furosemide.

The majority of patients (80 % before CB TNCs transplantation) received bisoprolol (*Concor*, Takeda, Japan) as a beta-blocker. carvedilol (*Kyiv Vitamin Factory*, Ukraine) was used in 20 % of patients with reduced ejection fraction (up to 20 %) due to alpha-adrenergic blocking effects. Angiotensin-converting-enzyme (ACE) inhibitors were used only in 50 % patients and in minimal doses, due to arterial hypotension. The main ACE inhibitor was ramiprilum (*Tritace*, Sanofi-Aventis S.p.A., Italy). For patients, who came to hospital with optimally prescribed doses of captopril (*Kyivmedpreparat*, Ukraine), the drug was not changed.

HF progression was not found in the patients, except two cases. Average doses of beta-blockers and ACE inhibitors remained relatively stable throughout the study: mean dose of bisoprolol was 2.4-3.1 mg/day, carvedilol – 6.25-7.8 mg/day, ramiprilum – 2.0-2.5 mg/day, captopril – 4.68-6.25 mg/day.

All patients with refractory HF had an increased cardiovascular disease mortality risk (see Tab. 4). Reducing both 1-year and 3-year mortality risk according to MAGIC score was registered since the first month of observation after CB TNCs transplantation. In the study, 1-year risk decreased by 10.1 %, 15.2 %, 37.4 %, 30.5 %, compared with the initial state by the end of the 1st, 3rd, 6th and 9th month of observation, respectively. Relatively to the initial state, 3-year risk decreased by 9.1 %, 26.6 %, 42.3 %, 31.8 % by the end of the 1st, 3rd, 6th and 9th months of observation, respectively. Reduction of the cardiovascular disease mortality risk, compared with the initial state, can provide an opportunity to extend waiting period for heart transplantation.

VALUE		OBSERVATION TIME, MONTH				
		0 (N = 20)	1 (N = 18)	3 (N = 18)	6 (N = 14)	9 (N = 14)
Functional class by NYHA	I	–	–	–	–	–
	II	–	6	12	12	9
	III	16	10	6	2	5
	IV	4	2	–	–	–
Average FC by NYHA		3.2	2.8	2.3	2.1	2.4

Table 3. Changes in heart failure functional class in patients with CB TNCs transplantation.

Notes: NYHA – New York Heart Association, FC – functional class.

Table 4. Changes in 6-min walk distance, the level of NT-proBNP, one- and three-year mortality risk by MAGIC score in patients before and after CB TNCs transplantation.

VALUE	INITIAL STATE, (N = 20)	AFTER CB TNCs TRANSPLANTATION			
		1 MONTHS (N = 18)	3 MONTHS (N = 18)	6 MONTHS (N = 14)	9 MONTHS (N = 14)
6-min walk distance, m	225.5 ± 72.5	272.0 ± 70.7	357.1 ± 44.4	376.4 ± 55.5 *	350.4 ± 64.4 *
Concentration of NT-proBNP in blood, pg/mL	2370.3 ± 448.9	2300.7 ± 403.0	1726.7 ± 432.1	1198.6 ± 396.3	1367.1 ± 381.1
1-year mortality risk (MAGIC score), %	9.9 ± 6.6	8.9 ± 6.0	8.4 ± 5.6	6.2 ± 4.5	6.9 ± 4.6
3-year mortality risk (MAGIC score), %	27.4 ± 10.6	24.9 ± 9.9	20.1 ± 10.2	15.8 ± 8.9	18.7 ± 9.4

Notes: NT-proBNP – N-terminal pro-brain natriuretic peptide, TNCs – total nucleated cells; * – $p < 0.05$ compared with the initial state.

CONCLUSIONS

Thus, heart failure manifestations significantly reduced after CB TNCs transplantation, starting from the first month of observation. It was shown in a significant improving exercise tolerance and HF functional class by NYHA. Clinical application of CB TNCs allowed to increase significantly the effectiveness of traditional HF drug treatment and reduce the dose of diuretic therapy. The reduction of HF was confirmed by a dynamic decrease of NT-proBNP levels and estimated cardiovascular disease mortality risk. Peak values of signs and objective evidence of HF decrease were detected in the 3rd and 6th months of observation. In the long-term follow-up (9-month) the rates of HF were significantly better than before CB TNCs transplantation, however, some negative trends were registered in peak values.

Therefore, transplantation of cord blood total nucleated cells can be an effective additional treatment for patients with refractory heart failure, who were added to a waiting list for heart transplantation.

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