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FETAL STEM CELLS IN COMBINED TREATMENT OF CHRONIC HEART FAILURE AND THEIR EFFECT ON MORPHOFUNCTIONAL PARAMETERS OF THE LEFT VENTRICLE MYOCARDIUM

ABSTRACT

Fetal stem cells treatment (FSCT) is assumed to be a new direction in a combined therapy for the patients with the severe chronic heart failure (CHF).

20 patients (average age of 50.1 ± 1.1 yrs.) with CHF functional class III-IV (FC) undergoing the transplantation of the cryopreserved cells of the fetal liver and heart obtained from 5-8 weeks gestation embryos were examined. The control group (CG) of 20 patients was compared by gender and age with the main group (MG).

Within 6 months after FSC therapy CHF patients reported a significant improvement of the contractile activity of the left ventricle (LV) heart muscle in echocardiography (*EchoCG*) findings (left ventricular ejection fraction (*LVEF*) increased by 20.9% being 2-fold higher than in patients without FSCT, $p<0.05$) and in features of LV remodeling (left ventricle end diastolic volume (LV EDV) decreased by 20.5%, $p < 0.05$). Serum *NT-proBNP* significantly raised within 1 month after FSCT by 33.8%, 50% and 65.1% in 1, 3 and 6 months respectively ($p<0.001$) and was significantly lower after a month of treatment compared with CG ($p<0.05$).

As a result of general condition improvement in CHF patients significant elevation in 6 minute walking distance (*6MWD*) test was observed and the distance walked increased 7.3-fold ($p<0.001$), 10.3-fold ($p<0.001$) and 12.5-fold ($p<0.001$) vs. the baseline in 1, 3 and 6 months, respectively, which was generally 2-fold higher than in patients of the CG. *DASI* score increased by 54.6%, after 3 months – by 63.2%, after 6 months – by 66.4%, which is significantly higher than the baseline ($p<0.05$ vs. baseline).

It has been proven that combined treatment of CHF patients using FSCs along with the standard therapy increases the LV myocardial contractility, lowering the blood serum *NT-proBNP* level and results in overall life quality improvement among the patients.

KEYWORDS: heart failure, fetal stem cells, left ventricle function remodeling.

Cardiovascular diseases are the leading cause of death worldwide and a great concern of healthcare institutions in all developed countries [20, 21]. According to the World Health Organization, cardiovascular diseases caused the death of 17.3 million people in 2008 – representing 30% of the general mortality worldwide. The importance of this problem differs in high- and low-income countries. In the high-income countries more than 80% of people die of cardiovascular diseases and this mortality rate is approximately the same between men and women. Until 2030, cardiovascular diseases, mainly cardiac illnesses and stroke as the sole cause of death, are expected to take lives of 23.6 million people each year [1, 26]. Chronic heart failure (CHF) is a result of any cardiovascular pathology.

CHF is caused by the reduced contractile power of the cardiac muscle, insufficient for normal blood outflow from the left ventricle (LV), which results in low supply of oxygen and nutrients [24]. Within the recent 10-15 years the approaches to chronic heart failure have changed. The new categories of medicines appeared, considerations about efficacy of inotrope substances changed as a consequence of the detailed study of neurohumoral regulation disturbances in CHF progression overall. CHF patients besides the symptomatic therapy, use special defibrillators for treatment [16], but, in spite of all medical advancements, the number of CHF patients is growing every year since the died out cardiac myocytes are hardly to be restored using a single «conservative therapy».

Continuous increase of cardiovascular mortality all over the world calls for the development of the principally new, affordable and effective therapies for CHF. Fetal stem cells (FCSs), as a part of combined treatment, can open a new page in the treatment of CHF. According to separate investigations data FSCs can differentiate into cardiomyocytes the number of which is being progressively decreased in CHF [11, 18]. As a result of combined treatment with FSCs use the myocardium muscle bulk increases, its contractile activity is improved contributing to the CHF patients overall well-being, life expectancy and quality are increased among these patients [3, 12, 15].

MATERIALS AND METHODS

20 patients (the main group – MG) with CHF functional class (FC) III-IV according to the New York Heart Association Functional Classification (*NYHA*) were studied **(Table 1)**. CHF diagnosis was made on the basis of clinical symptoms (weakness, shortness of breath, edema of the lower extremities, palpitation, sleep apnea) confirmed by the findings of physical examination, laboratory test results (serum *NT-proBNP* increase) and echocardiography (*EchoCG*) findings (*LVEF* < 45% and increased end diastolic volume (EDV) > 140 ml).

The causes of CHF included ischemic cardiomyopathy (14 patients, 70%), alcohol-induced cardiomyopathy (3 patients, 15%) and dilatation cardiomyopathy (3 patients, 15%). The MG consisted of 20 patients, including 14 men (70%) and 6 women (30%), and their age ranged between 33 and 60 (mean 50.1 ± 1.1) years. Average disease and CHF treatment history were 7.6 ± 0.5 and 6.3 ± 0.6 years, respectively. The number of CHF patients with *NYHA* FC III and FC IV were 16 (80%) and 4 (20%), respectively.

The control group (CG) included 20 CHF patients whose age and sex was similar to the study group. Among the patients in CG, CHF was caused by ischemic cardiomyopathy – 16 (80%), dilatation cardiomyopathy – 3 (15%) and alcohol-induced cardiomyopathy – 1 (5%). The number of patients with *NYHA* FC III and FC IV CHF were 14 (70%) and 6 (30%), respectively **(Table 1)**.

All patients were undergoing routine CHF therapy in accordance with recommendations of the European Society of Cardiology (2011) for a long time: diuretics (torasemide – average dose 12.5 ± 1.3 mg, beta adrenoblockers (carvedilol – average dose 46.9 ± 7.9 mg), angiotensin converting enzyme inhibitors (perindopril arginine – average dose 6.5 ± 0.7 mg) and/or angiotensin receptor blockers (ARBs) (valsartan – average dose 100 ± 10.5 mg), cardiac glycosides (digoxin – average dose 0.19 ± 0.01 g), and also indirect anticoagulants (warfarin – average dose 4.8 ± 0.4 mg). All prescriptions were individual **(Table 2)**.

Fetal stem cell treatment (FSCT) was carried out in combination with standard therapy for patients selected from the main group. No patient among the main and control groups suffered from infections, malignancy or mental diseases.

In FSCT, cryopreserved suspension containing pluripotent FSCs harvested from 5-8 weeks old legally aborted embryonic cadavers was used. For this purpose all women according to social indications were suggested to sign the informed consent for abortion material use. Aborted tissues were collected pursuant to ethical, moral and legal principles of work with biological tissue. All donors were healthy women with negative test results for hemic infections.

The biotechnological process of suspension preparation included cell harvesting from germ layers (liver, brain, heart and soft embryo tissues), viability testing, programmed cryopreservation, bacterial and viral safety testing. Water bath thawing of cryopreserved suspension at 37 °C and viability testing were performed before FSCT.

Cryopreservation was performed with 5% dimethyl sulfoxide in 3 stages with an initial temperature program of 1 °C/min and crystal forming initiation. Pre-administration cell viability was tested by trypan blue staining. Cells were counted in parallel, in Goryaev chamber and in an automated cell counter *TC10™* (*Bio-Rad*, USA). The proportion of live cells in the suspension was found to be 83.0 ± 3.0%. After the preservation in a low temperature bank (t° = -196 °C) and further water bath heating at temperature +37.5 ± 0.1 °C viability of cells was not less than 74.8 ± 1.03%.

The patients were treated during 2 days with FSCs: for the first day drip intravenous injections of fetal liver cells were used for the patients, the cells of fetal heart were administered in the anterior abdominal wall during the second day of treatment. Therapeutic doses were individually selected for each of transplantations, but were not below 0.1 ml of cells suspension containing not less than $0.1 \cdot 10^8$/ml of nucleated cells and $0.3\text{-}2.54 \cdot 10^6$/ml of $CD34^+$ precursors per transplantation. The proportion of living cells in the suspension was 80.0 ± 10.0%.

All the patients signed their informed consent having been examined before treatment and repeated clinical examination was performed in 1, 3 and 6 months after treatment. *DASI* (Duke Activity Status Index) scale was used for physical capacity evaluation of the patients. The patients underwent 12-lead ECG, Echo Doppler, 6-minute walking distance test and *NT-proBNP* test.

A *Cardiovit CS-100* cardiopulmonary cart instrument (*Schiller Medical*, Switzerland) was used for ECG and to record rhythm and conduction irregularities, features of LV hypertrophy, *ST* segment ischemia, *Q*-wave pathology, non-specific irregularities of *ST* segment and *T*-wave.

Table 1. Distribution of the patients into the main and control groups, depending on the NYHA FC.

NYHA IV FC		NYHA III FC	
MG	CG	MG	CG
4 (20,0%)	6 (30,0%)	16 (80,0%)	14 (70,0%)

Table 2. Routine treatment of CHF patients depending on NYHA Functional Class (FC)

PHARMACEUTICAL	MAIN GROUP		CONTROL GROUP	
	FC IV	FC III	FC IV	FC III
warfarin, n	12 (60,0%)	4 (20,0%)	11 (55,0%)	6 (30,0%)
aspirin, n	4 (20,0%)	-	3 (15,0%)	-
carvedilol, n	16 (80,0%)	4 (20,0%)	14 (70,0%)	6 (30,0%)
torasemid, n	10 (50,0%)	4 (20,0%)	9 (45,0%)	6 (30,0%)
perindopril, n	10 (50,0%)	2 (10,0%)	10 (50,0%)	5 (25,0%)
valsartan, n	6 (30,0%)	2 (10,0%)	4 (20,0%)	1 (5,0%)
digoxin, n	5 (25,0%)	4 (20,0%)	6 (30,0%)	6 (30,0%)

Doppler *EchoCG* was performed on *Toshiba SSA 380A Powervision 7000* (*Toshiba Corp*., Japan) with variable frequency (2.5–3.5 MHz) in supine and on the left side in accordance with the routine procedure. Systolic and diastolic functions of heart ventricles and their remodeling were also controlled [5].

The 6-minute walking distance (*6MWD*) test, expressed in meters, was carried out and compared with baseline *6MWD* (i). *6MWD* (i) was calculated by the formula below with respect to the patient's age (years) and body mass index (BMI):

- for Men 6MWD (i) = 1140 - 5.61 x BMI - 6.94 x age,
- for Women 6MWD (i) = 1017 - 6.24 x BMI - 5.83 x age,

where BMI is body weight (kg)/height2 (m) ratio.

The presence of NT-proBNP in the serum is indicative of early stages of cardiac failure and the most demonstrative marker of ventricular dysfunction. Its level correlates with CHF stage. An enzyme-immunoassay was used for *NT-proBNP* testing [8].

Significant differences between mean values were calculated by Student's t-test (for parametric statistics) and Mann-Whitney test (for independent samples). The difference was regarded as statistically significant if $p<0.05$. The data were processed using software *Statistica 8.0* (*StatSoft Inc*., USA).

RESULTS AND DISCUSSION

Within a month after FSCT, all MG patients with *NYHA* FC IV (4 patients or 20%) were downgraded to *NYHA* FC III and 5 patients with *NYHA* FC III (25% from the total number or 1/3 of patients with *NYHA* FC III) – to *NYHA* FC II. Thus, 1 month after FSCT, none of the patients were classified *NYHA* FC IV CHF, while 15 and 5 patients manifested *NYHA* FC III (75%) and *NYHA* FC II (25%) CHF, respectively. Three months after FSCT, the number of patients with *NYHA* FC III CHF was reduced by 4 (to 11 patients), and 9 patients showed *NYHA* FC II CHF. Six months after FSCT, 8 and 12 patients scored *NYHA* FC III (40%) and FC II CHF (60%), respectively.

As for CG, within a month after FSCT, 3 patients with *NYHA* FC IV (15% from the total number of FC IV patients) were downgraded to *NYHA* FC III and 4 patients with *NYHA* FC III (20%) – to *NYHA* FC II. Three months after FSCT, 3 remaining patients with *NYHA* FC IV CHF were downgraded to *NYHA* FC III, the number of *NYHA* FC III patients was reduced by 3 (to 13 or 65% patients), 7 (35%) patients showed *NYHA* FC II CHF and none had *NYHA* FC IV CHF. Six months after FSCT, 11 (55%) and 9 (45%) patients scored *NYHA* FC III and FC II CHF respectively. The difference in the number of patients with *NYHA* FC II, III and FC IV one, three and six months after FSCT was insignificant in both MG and CG, however the tendency for *NYHA* FC downgrading and its rate can be clearly traced **(Table 3)**.

The next phase of the study was defining changes of CHF patients' life quality on *DASI* scale, physical tolerance (*6MWD*) and morphofunctional parameters of LV myocardium **(Table 4)**.

The treatment resulted in the improvement of the studied parameters: increase of *DASI* score, physical tolerance (*6MWD*) and *LVEF* while EDV in the LV was reduced accordingly.

Within a month after FSCT, MG *DASI* score increased by 54.6%, after 3 months – by 63.2%, after 6 months – by 66.4%, which is significantly higher than the baseline ($p < 0.05$ vs. baseline). At the same time, CG *DASI* score increased by 10.7% one month after the treatment, in 3 months – by 38.1% ($p < 0.05$) and in 6 months – by 41.7%, which is significantly higher in comparison with baseline ($p < 0.05$). Comparative analysis of the results in both groups revealed that within a month after the treatment significant increase of *DASI* score in MG was 5-fold higher than in CG and 6 months after the treatment significant difference between the groups was preserved ($p < 0.05$) – it was 30% higher in MG. Thus, in both groups, functional capacity index significantly improved over the 6 months after the treatment, but the improvement rate was quicker (1 month after the treatment) and more intensive in the group that underwent FSCT.

In MG, significant changes on *6MWD* were reported as early as 1 month after the combined treatment with FSC the distance walked

Table 3. NYHA FC dynamics on the background of routine treatment with/without FSCs

OBSERVATION TERM	NYHA CLASS IV		NYHA CLASS III		NYHA CLASS II	
	MG	CG	MG	CG	MG	CG
Before treatment	4 (20,0%)	6 (30,0%)	16 (80,0%)	14 (70,0%)	0	0
1 month	0	3 (15,0%)	15 (75,0%)	13 (65,0%)	5 (25,0%)	4 (20,0%)
3 months	0	0	11 (55,0%)	13 (65,0%)	9 (45,0%)	7 (35,0%)
6 months	0	0	8 (40,0%)	11 (65,0%)	12 (60,0%)	9 (45,0%)

Table 4. Changes in integrated clinical evaluation and morphofunctional parameters of the LV myocardium in CHF before and after FSCT in the main and control groups

TEST	MG before treatment	CG before treatment	MG, 1 month	CG, 1 month	MG, 3 months	CG, 3 months	MG, 6 months	CG, 6 months
DASI , PTS (M±m)	15,2 ± 1,85	15,4 ± 1,48	23,5 ± 3,4$^{*\#}$	16,8 ± 0,4*	24,8 ± 3,4*	21,0 ± 1,2*	25,3 ± 3,4$^{*\#}$	21,5 ± 0,3*
6 MWD, M, (M±m)	30,0 ± 4,5	31,5 ± 4,9	220,5 ± 7,4$^{\alpha\beta}$	167,4 ± 6,2$^{\alpha}$	325,0 ± 1,2$^{\alpha\beta}$	213,75 ± 10,3$^{\alpha}$	375,0 ± 13,6$^{\alpha\beta}$	285,3 ± 7,0$^{\alpha}$
LVEF,%, (M±m)	36,8 ± 2,9	36,1 ± 2,9	37,1 ± 2,9	35,9 ± 0,3	40,5 ± 2,4*	38,8 ± 0,3	44,5 ± 1,6$^{*\$\#}$	40,2 ± 0,3$^{*\$}$
EDV, ML, (M±m)	222,8 ±19,3	225,1 ± 17,6	205,3 ± 10,1	212,6 ± 3,5	192,7 ± 7,6	205,0± 3,7	177,1 ± 11,3$^{\alpha\$}$	200,4 ± 1,9$^{\alpha\$}$

Note: * – $p < 0.05$ compared with the group before the treatment; α – $p < 0.001$compared with the group before the treatment; $ – $p < 0.05$ compared with the groups 3 months after the treatment; # – $p<0.05$ compared with the control group; β – $p < 0.001$ compared with the control group.

increased 7.3-fold ($p < 0.001$), 10.3-fold ($p < 0.001$) and 12.5-fold ($p < 0.001$) in 1, 3 and 6 months, respectively. In CG, test results also improved as the distance walked increased 5.6-fold ($p < 0.001$), 7.1-fold ($p < 0.001$) and 9.5-fold ($p < 0.001$) in 1, 3 and 6 months, respectively. Significant difference between *6MWD* score between the groups was obvious within a month after the treatment: in MG, the result was higher by 24.1% ($p < 0.001$), 34.2% ($p < 0.001$) and 23.9% ($p < 0.001$) in 1, 3 and 6 months respectively.

In MG, *LVEF* remained almost unchanged during the first month after FSCT. However, it increased by 10.5% ($p < 0.05$) and by 20.9% ($p < 0.05$) in 3 and 6 months after FSCT, respectively, in comparison with the baseline and by 9.9% ($p < 0.05$) in comparison with 3-months result. In CG, *LVEF* remained practically unchanged within 1 and 3 months vs. baseline and this parameter of MG patients. After 6 months, it increased by 10.1% in comparison with the baseline ($p<0.05$), but it was 9.6% lower in comparison with MG ($p < 0.05$).

In MG, LV EDV decreased as early as within a month after FSCT, and continued to drop afterwards – by 7.9% ($p < 0.05$), 13.5% ($p < 0.01$) and 20.5% ($p < 0.01$) in 1, 3 and 6 months, respectively. In CG, LV EDV decreased by 9.1%, 12.5% and 17.7% ($p < 0.05$) in 1, 3 and 6 months after the treatment, respectively. Moreover, significant difference ($p<0.05$) between the groups was obvious 6 months after the treatment, in the average by 23.3%. In MG, LV EDV reduction was more intensive than in CG, though insignificant.

In MG, serum *NT-proBNP* significantly decreased within 1 months after FSCT – (by 33.8%, 57.2% and 65.13% in 1, 3 and 6 months respectively ($p < 0.001$ for all) (Figure 1). Routine treatment also resulted in *NT-proBNP* reduction by 15.3%, 44.9% and 64.9% in 1, 3 and 6 months respectively vs. baseline, $p < 0.001$ for all (Figure 1). It is obvious that serum *NT-proBNP* in MG decreases at a quicker rate than in CG – by 21.2%, 27.8% and 18.5% in 1, 3 and 6 months, respectively ($p < 0.05$ for all). Thus, with routine therapy, *NT-proBNP* decreased in both groups, however in MG it was significantly higher starting from month 1 and throughout all the observation period.

Clinical improvements, both objective and subjective, were reported in all CHF patients. Significant increase of *LVEF* by 20.9% ($p < 0.05$) and LV EDV reduction by 20.5% ($p < 0.05$) were reported 6 months after FSCT. *NT-proBNP* was reduced by 33.8%, 50.0% and 65.1% ($p < 0.001$ for all) in 1, 3 and 6 months after FSCT. The *6MWD* test result increased 7.3-fold, 10.3-fold and 12.5-fold after 1, 3 and 6 months after FSCT ($p < 0.01$ for all). Within a month after treatment, *DASI* increased 2-fold, and after 6 months by 66.4% ($p < 0.01$ for both).

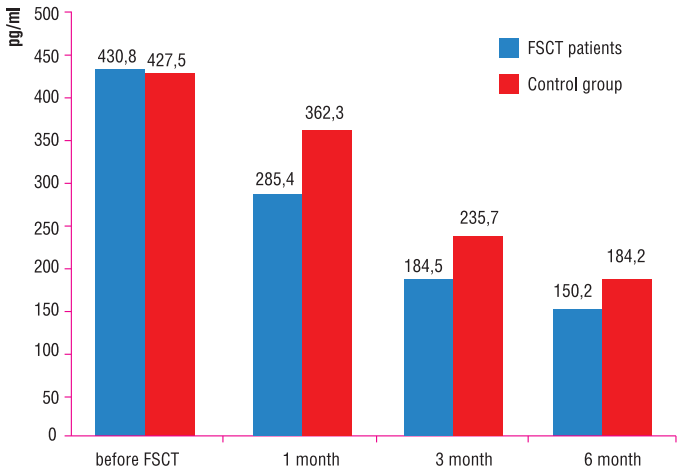

Fig. 1. *NT-proBNP* dynamics during treatment (see data comparison in the text)

Although a 6-month follow-up period is far not sufficient for recommendations regarding wide clinical application, it is notable, that no side effects affecting the cardiovascular system or brain function have been reported during the entire period of observation of CHF patients treated routinely in combination with FSCT. Clinically there were no cases of treatment-induced allergy. Thus, the present FSCT method can be regarded as safe for further long-term observations.

In CHF, FSCT was used for improvement of heart contractile activity and LV remodeling processes regulation through substitution of the cells that are unable to contract, necrotic or sclerotic tissues. In our opinion, functional restoration of the myocardium can be reached through the increase of the number of cells capable of contraction in myocardium and/or through increasing functional reserve of the patient's cardiomyocytes via stimulation of intracellular regeneration processes [4, 6, 14].

It is common knowledge that cardiomyocytes are electromechanically integrated through special contacts in the area of the intercalated discs of cardiac muscle containing *fascia adherens* (myofibril adherence area), gap junctions and desmosomes [1, 19, 22]. Desmosomes and *fascia adherens* perform mechanical function, or, in other words, fix cardiomyocytes, while gap junctions conduct electric impulses [9, 17]. Electromechanical integration of cardiomyocytes is of paramount importance for coordinated activity of myocardium making heart muscle work as an integral unit. The above taken into consideration, the success of cardiomyocyte transplantation is defined by the fact whether it will be capable to interact with the muscle cells of the recipient and microenvironment [2, 7, 23].

The experimental use of human fetal tissues is a very sensitive and controversial issue. It is not supported and even is prohibited in certain countries, including the USA and the EU. In other countries, including Ukraine, this biological material can be used for experimental purposes upon the consent of the woman making decision about the abortion and donation of the fetal material in accordance with ethical, moral and legal principles. There are many opponents of the use of the aborted human embryonic tissues for medical purposes, because, in their opinion, this will result in a growing "demand" for abortions. In our emancipated times, when most women are working, holding top positions, they wish to plan their life and decide about the timing of childbirth. Therefore, in spite of the protests of some political and religious organizations, abortions will not stop. Taking into consideration the value of fetal material with its huge potential of helping many people, even with the incurable diseases, it would be inhumane not to use this opportunity to save or improve people's lives. With the view to small, but very promising experience of the use of FSCs capable of differentiation into different functional specialized cells, scientists lay great hopes for effective clinical use of these cells.

Apart from the above, some experts fear undesirable adverse effects of FSCT. According to the literature [13, 27], wrong differentiation of embryonic stem cells can result in tumor (teratoma) formation. In our study, we used fetal stem cells that, unlike embryonic cells, do not have potential oncogenic properties, and no one such carcinogenic case was reported by the patients. Another discussed adverse effect of FSCT is rejection; however, due to immaturity of *HLA* receptors in 5-8 weeks old fetuses [10, 25] rejection does not develop. No similar phenomenon was reported by any of the patient according to the general clinical criteria. Therefore, our preliminary conclusion is that routine therapy in combination with FSCs is a promising and safe method of CHF treatment.

The results obtained bring hope for combined treatment of CHF with routine methods and FSCT, and possibly allow FSCT as a future alternative to heart transplantation. Obviously, this concerns, first of all, the patients on waiting lists for heart transplantation. It is too early to make any assumption regarding the potential role of FSCT in prevention of cardiomyocyte apoptosis, but there is hope that trials in progress will shed light on this aspect of FSCT effect on the course and prognosis of CHF.

CONCLUSIONS

1. COMBINED CHF TREATMENT WITH ROUTINE METHODS AND FSCS AFTER 6 MONTHS OF THERAPY RESULTS IN IMPROVED CONTRACTILE ACTIVITY OF THE MYOCARDIUM (LEFT VENTRICLE EJECTION FRACTION INCREASE IS 2-FOLD HIGHER VS. BASELINE IF COMPARED WITH THE CHF PATIENTS WHO WERE TREATED WITHOUT FSCT) AND LV REMODELING (LEFT VENTRICLE END DIASTOLIC VOLUME DECREASE VS. BASELINE). CONSEQUENTLY AGAINST THE BACKGROUND OF STANDARD THERAPY BLOOD SERUM *NT-proBNP* LEVELS TEND TO DECREASE IN BOTH GROUPS, HOWEVER, BEGINNING FROM THE 1 MONTH THE DECREASING RATE WITHIN THE MG WAS SIGNIFICANTLY HIGHER AND THIS DYNAMICS WAS PRESERVED DURING THE WHOLE PERIOD OF OBSERVATION.

2. CHF PATIENTS REPORTED SIGNIFICANT FUNCTIONAL AND LIFE QUALITY IMPROVEMENTS AFTER 1, 3 AND 6 MONTHS OF FSCT WHICH WAS DEMONSTRATED BY THE *6MWD* AND *DASI* SCORES INCREASING, RESPECTIVELY.

3. IT HAS BEEN PROVEN THAT FSCT IS A SAFE AND EFFECTIVE COMBINED THERAPEUTIC METHOD THAT CAN BE USED ALONG WITH THE CONSERVATIVE TREATMENT IN ADVANCED CHF. THIS METHOD OFFERS GREAT HOPE, BUT, AT THE SAME TIME, CALLS FOR CONTROLLED RANDOMIZED CLINICAL TRIALS.

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