

Autologous Stromal Stem Cell Infusion in Idiopathic Dilated Cardiomyopathy

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Regenerative medicine, particularly stem cell therapy, has found its application in the cardiovascular field. For patients with cardiomyopathies, several case studies have been done and there are ongoing trials, particularly using intramyocardial or intracoronary route of stem cell administration.

We report two cases of patients with non ischemic dilated cardiomyopathy, wherein despite optimized medical therapy, they are symptomatic. Unlike other approaches in the cardiomyopathy therapy, we utilized only purified bone marrow mesenchymal cells instead of total bone marrow for them. Moreover, these purified bone marrow mesenchymal cells were infused instead of injected. Both patients responded well to stem cell infusion. There were improvements in echocardiographic parameters of cardiac function, which were highest after the third infusion. Decline in ejection fraction were noted after an infectious process. The first patient is currently alive (survived for at least 4 years from the time of infusion); while, the second patient succumbed to sepsis 9 months from the stem cell infusion.

Various factors may affect the success of cell therapy: specific type of stem cells from a specific source, amount of cells to be utilized, appropriate mode of administration and others external factors. This is a pioneering approach of intravenously administered autologous bone marrow derived mesenchymal stromal stem cells utilizing minimally invasive technique through slow infusion. This technique reduces endothelial injury and arrhythmogenicity where the overall observation of this study suggest safety and feasibility, along with our clinical outcome leads us to convey this alternative therapy has a role in cardiac regeneration of dilated cardiomyopathy non ischemic type. This report may serve as a guide for further search of this new individualized therapeutic strategy of treatment. *Phil Heart Center J 2012;16:1-11*

Key Words: Stem Cell ■ Regenerative Medicine ■ Dilated Cardiomyopathy ■ Autologous Stromal Cell Infusion

Dilated Cardiomyopathy is defined by the World Health Organization in 1995 as a “disease condition that effects cardiac dilatation and impaired contraction of one or multiple ventricles.”¹ The overall incidence as determined by US statistics is 1-2 cases per 100,000 people. In a study by the National Heart, Lung and Blood Institute, they determined that it is responsible for 10,000 deaths and 46,000 hospitalizations annually and contributed to one third among the causes of heart failure with a mortality rate of 53 % per year in the United States.² Cardiac transplantation is still the ultimate and definitive management; however, coupled with the worldwide dilemma of organ scarcity, left ventricular assist device had been utilized as

as a temporary treatment modality as a bridge to transplantation surgery. Even if these cardiac assist devices have a high acquisition cost and advantageous for short term administration only, its availability especially in developing countries would benefit even a small percentage of population with such debilitating disease.³ Thereby, a newer approach to address various problems can be made through autologous cell therapy. Regenerative Medicine finds its interventional application in dilated cardiomyopathy through advances in technology and molecular understanding of the disease process. Cellular Transplantation is an innovative treatment of various disease conditions. In this instance, it is for the purpose of myocardial regeneration

in end stage heart disease. Stem cells derived from the patient's own bone marrow cells has its advantage in eliminating the possibility of rejection and avoiding cytotoxicity of immunosuppression. In a review by Ye Chen et al, they said that the bone marrow is the richest and most reliable source for mesenchymal cells (MSC), which exhibit multipotent differentiation potential and have been shown to reconstruct to different mesodermal cells.⁴ An original article by Arguero et al. reported that autologous stem cells obtained through hemophoresis and administered by intramyocardial injection can cause an improvement of ejection fraction.⁵ Unlike other approaches in the cardiomyopathy therapy, we utilized only purified bone marrow mesenchymal cells instead of total bone marrow. Moreover, these purified bone marrow mesenchymal cells were infused instead of injected. We report two cases of non ischemic dilated cardiomyopathy and the effect of stromal stem cell therapy on this severe condition. Our aim is to report such treatment for severe heart failure that gives an option to aid in longevity with good quality of life.

CASE REPORT A

Our patient is a 44 year-old male police officer, (74 kg, 175cm) who presented with progressive dyspneic symptoms for four months. Personal and previous medical history was unremarkable except for a significant smoking history of 28 pack years. On physical examination, blood pressure (BP) was 110/50 mmHg cardiac rate (CR) was 103 beats per minute, respiratory rate (RR) 20 breath per minute. Cardiac auscultatory findings showed a dynamic precordium with an apex beat displaced to 6th LICS left anterior axillary line; Grade 3/6 holosystolic murmur and S3 gallop. Lung fields were clear. Chest radiography showed cardiomegaly and 2D Echocardiography confirmed the impression of dilated cardiomyopathy with a 22% ejection fraction, left ventricular dilatation and mitral regurgitation. He had been on optimum medical treatment with Furosemide 40mg/tab ½ tablet OD; Spirinolactone 25mg every other day; Enalapril 20mg/tab OD; Carnitine 375mg OD; Digoxin 0.25mg ½ tab OD; and Metoprolol 50mg/tab OD which afforded no relieved of symptoms.

On admission, blood chemistry showed an elevated lipid profile. Electrocardiography demonstrated sinus rhythm with left bundle branch block. Coronary angiography results validated the diagnosis of non ischemic dilated cardiomyopathy. After thorough discussion, informed consent was obtained when the family decided on stem cell therapy.

Forty (40) ml of bone marrow cells aspirated from posterior iliac crest were transported to molecular laboratory for processing. An equal volume of RPMI condition medium was added and centrifuged at 5000RPM for 15 minutes using a Ficoll-hypaque suspension. Afterwards, it was resuspended in RPMI medium together with patient's serum utilizing CO2 incubator for cell growth with highly individualized monitoring technique until differentiation occurs. The combination of filtered stem cells with conditioned medium and granulocyte colony stimulating factor make up the stem cell preparation.

After incubating and mixing of the stem cell preparation, it was slowly infused over 30 minutes under cardiac monitor. During the first infusion, the patient experienced an occasion of slight midsternal heaviness on the 20th minute manifested as widened QRS on ECG for 5 seconds that resolved spontaneously. There was also breast tenderness noted that subsided after two months without treatment. However, no similar observation was noted on succeeding infusion. There were occurrence of generalized warm sensation and slight agitation after every infusion. The warmth phenomena spontaneously disappeared in 12 hours. Vital signs recorded post infusion: blood pressure 100-120/50-60 mmHg; cardiac rate 60-103 beats per minute; respiratory rate 18-20 breaths per minute; Oxygen saturation 95-100%; and temperature 36.5 – 37°C. He was discharged after 24 hours of ICU observation. He received a total of seven infusions in three years. Effectiveness of therapy was measured by parameters on 2D echocardiography, tapering of maintenance medications and quality of life assessment.

Ejection fraction determined by 2D echocardiography using Simpson's Method two to three months after infusion showed improvement in four out of seven infusions. However, there was no significance improvement as far as function is concerned as shown in left ventricular end

systolic diameter. Ejection fraction showed an initial surge of 26.5% (35% using Simpsons Method) and an average enhancement of 26% (31% using Simpson's Method). Right heart function improved with normal contractility; however, the left sided function remained unchanged.

There was initial favorable response in mitral valvular function, albeit, there was persistent mitral regurgitation due upper respiratory tract infection after the second infusion. Metoprolol 50mg was discontinued after the first infusion secondary to controlled cardiac rate. Physical activity as measured by MET score based on Philippine Heart Center Comprehensive Cardiac Rehabilitation Program classification of Activities improved from an initial score of 3-4 to 7-8.

Stress ECG done at the end of 6th cycle infusion showed a METS score of 4. He had been back to work as police officer after every infusion with good quality of life and an average cardiac output of 8.1L/ml.

CASE REPORT B

This is a case of a 45 year old male (72kg, 170 cm.) with history of syncope accompanied by loss of speech and right hemiparesis. His chief complaint was difficulty of breathing and easy fatigability. Physical examinations showed gallop rhythm with jugular venous distention, clubbed fingers and cyanotic nail beds. He was classified under NYHA Classification III-IV.

Diagnostic procedures done revealed cardiomegaly on radiography; conduction defect with average ventricular response on 24 hour Holter report; and multi-chamber dilatation with eccentric left ventricular hypertrophy on 2D echocardiography. The latter confirmed the diagnosis of dilated cardiomyopathy. Furthermore, a normal coronary angiography rule out ischemic cause and directed us to its idiopathic origin.

Several palliative interventions were instituted and included biventricular pacemaker insertion to achieve rate control of intraventricular conduction defect, which was done thirteen years ago; left atrial thrombectomy and mitral valve replacement performed three years ago to

alleviate symptoms of mitral regurgitation and retard progression of pulmonary hypertension along with various therapeutic combination of diuretics, digitalis, ACE inhibitor, angiotensin II receptor antagonist, beta blocker, vasodilator and antithrombotic. All efforts to retard the progressive effect of this condition had been attempted and had benefited him for more than a decade. Pacemaker interrogation done three years ago showed good capture. Despite the convergence of all these effects, cardiac function improve for sometime and revert back to poor myocardial contractility based on echocardiography reports.

With the advent of cellular transplantation, recent reports showed remarkable effect of stem cell therapy on affected myofibrils. Various derivation and methodologies have been reported. In our patient, we utilized bone marrow aspirated through iliac crest under local anesthesia and obtained 80 -100 cc of bone marrow cells. The collected specimen was then processed in the laboratory that involved separation of white blood cells from red blood cells. The white blood cells were cultured and grown under carbon dioxide incubator. Individualized cell differentiation and thrombocytes removal are the unique features of our stem cell preparation, which was administered through minimal invasive approach via intravenous infusion. This technique minimizes the occurrence of arrhythmogenicity that is detrimental in a severely compromised myocardium.

During the first infusion, despite all the contingency preparation on premedication and sensitivity test of the actual stem cell preparation, the patient had an anaphylactic reaction characterized by generalized chills and temperature elevation leading to rapid ventricular response and hypotension at 40th minute after infusion. The effect was managed with pressors, antihistamine, and corticosteroids. He was maintained on ICG non invasive hemodynamic monitor until the effect subsided the following day. He was discharged from the hospital after two observation days and had resumed employment in a week. Two-dimensional echocardiography was utilized for outcome assessment. A repeat examination done after a month showed an improved ejection fraction from 38% to 48%. Furthermore, the previously dilated main pulmonary artery with mild pulmonary arterial hypertension fairly subsided

and achieved a normal pulmonar artery pressure.

He underwent a total of seven (7) infusions on a monthly basis. There was improvement of the ejection fraction after the initial infusion to 43%. Ejection fraction reached the normal acceptable level after the third infusion. Pulmonary arterial hypertension subsided after the second infusion.

After the third infusion, 2D echocardiography, in terms of hemodynamic evidence, showed an improved cardiac output and stroke volume with an ejection fraction of 57%. Left ventricular end systolic diameter had improved as well. Combined with results taken from the BIOZ ICG, a non invasive hemodynamic monitor to assess the functional capacity of myocardium, there is normal myocardial contractility indices, although it is in the lower limit of normal. Thoracic fluid content was likewise normal and is within the upper limit of normal value.

There was noticeable decrease in ejection fraction after every upper respiratory tract infection and gastrointestinal tract infection; otherwise, there were no noted serious adverse reaction.

Physical endurance to brisk walking improved as evident by his ability to walk without assistance through the airport (approximately 1-2 kilometers) without observable symptoms. He had been back to work after every infusion with good quality of life as well. His average cardiac output is 8.8 L/min.

He was able to complete the six (6) cycles of treatment. He had been well until he accidentally ingested unpasteurized milk that caused the constellation symptoms of abdominal infection and septicemia. He was able to recover from the insult and went back to work for another four months. He suddenly developed generalized tonic-clonic seizure with upward rolling of eyeballs. A repeat 2D echocardiography showed the presence of atrial thrombus. He responded to antithrombotic therapy and had regained consciousness; however, septicemia progressed rapidly that cause hypotension and finally, renal shutdown. He finally succumbed to all treatments.

DISCUSSION

Stem cells, which are early stage cells that pro

vide the stem to which other cells differentiate into other cell types, divide continuously and differentiate into other specialized cell types.⁶ Stem cells are undifferentiated cells with no specific physiological function; however, under proper conditions, they can develop into specialized tissues and organs with great abilities to turn into more specialized cells that were equipped with self sustained capability.

Adult bone marrow (BM) contains various cell population that display plasticity and regenerative capability.⁷ In a review, it was stated that the bone marrow is the richest and most reliable reservoir for mesenchymal stem cells (MSCs) as well as hematopoietic stem cells (HSCs), multipotent adult progenitor cells (MAPCs) and endothelial progenitor cells (EPCs).⁸ Mesenchymal or stromal stem cells (MSCs) are second generation of nonhematopoietic cell population in BM with estimate incidence ranges from 1:200,000 bone marrow mononuclear cells. Each stem cell population is characterized by its specific surface phenotype and its ability to differentiate into multiple cell types. Experiments showed MSCs capability of soluble factor production for homing and tethering and ability to avoid detection by the immune systems made it a prime candidate for cell therapy.⁹⁻¹⁰ Badoo and associates stated that these soluble factors, including hepatocyte growth factor (HGF), transforming growth factor-1, interleukin-1 (IL-1), IL-1 β , IL-3, IL-6, IL-7, IL-11, stem cell factor, and Fms-like tyrosine kinase 3 ligand, may enhance regenerative ability, stimulate proliferation and differentiation, and decrease inflammatory and immune reaction.¹¹ HGF causes re-organization of cytoskeletal protein, which have mechanical function to strengthen plasma membrane during heart muscle contraction and play an important role in signal transduction.¹²

One unexpected finding in MSCs was that it migrates to sites of tissue injury.¹³ This indicates that stem cells involved in tissue repair migrate even if initially present at distant site of injury. Homing, in bone marrow transplantation therapy, refers to the "phenomena by which intravenous stem cells specifically engraft in the bone marrow and not in other organs."¹⁴⁻¹⁵ Methodologies to monitor repair at cellular level, to identify signals for stem cell mobilization and trafficking to affected area of tissue involvement

based from recent work by John Hopkins colleagues showed MSCs are first deposited in the lungs for 24 hours, migrate to the infarcted area of the heart and persisted for a week. Cultured MSCs readily adhere to culture dishes forming fibroblast like colonies. It can be expressed in surface receptors such as CD 34+, CD 45-, CD 31-, CD 90+, and CD 106+ in humans.

There had been several studies regarding the differentiation capacity of different populations of bone marrow-derived stem cells into cardiomyocytes and data suggests that only MSCs seem to form cardiomyocytes.¹⁶ MSCs were endowed with the ability to alter the tissue microenvironment via soluble factors secretion that contributes significantly for transdifferentiation in tissue repair.¹⁷ Human MSCs were first isolated by Arnold Caplan and colleagues at Case Western Reserve University from small bone marrow sample drawn through the skin under local anesthetic.¹⁸ Injured myocardium leads to loss of cardiomyocytes and reduced heart function.¹⁹ To be therapeutically effective, bone marrow stem cells that transform to cardiomyocytes should be in sufficient amount to repair damage tissue and exhibit plasticity.²⁰

Aggarwal and associates²¹ had observed that the immunomodulatory effects of MSCs were through cell interaction from dendritic cell, natural killer cell and T-cell pathways. MSCs alter cytokine production such that pro-inflammatory DC-1 decreases secretion of tumor necrosis factor α and anti-inflammatory DC-2 increase IL-10 secretion. It also caused pro-inflammatory T helper1 cells to decrease secretion of interferon γ and cause anti-inflammatory T helper 2 cells to increase secretion of IL-4.

Mechanisms that responsible for the differentiation phenomena is unclear; potential possibilities include transdifferentiation, dedifferentiation, fusion with resident cells or a combination of these mechanisms.²²

Upon cell entrance in the circulating blood, adhesion molecules expressed at injury site mediate endothelial attachment. Chemokines mediated transendothelial migration leads to tissue directed differentiation through cell-to-cell contact and growth factors. Several investigators suggested that selective expression of organ-specific chemokines promotes the mobilization of bone-marrow-derived cells for tissue vascula-

rization and organ regeneration.²³⁻²⁶ Despite intensive investigation, the conversion pathways from mechanical signals to biochemical responses are not completely understood.²⁷ "chromatin shifts and cell cycle effects stem cell gene expression and changing cell cycle transit determine the regulation of stem cell growth and differentiation in the chaotic dynamics of transcriptional networks within a cell."²⁷ The process involves both cooperation and competition of chemokines and cytokines in the environment, and mechanical forces, such as: stretch, strain and laminar flow.

In ischemic cardiomyopathy, the natural course of infarction healing and the presence of putative homing signals within the damaged myocardium appear to favor cell engraftment during the transendothelial passage.²⁸ However, due to the high oxidative stress of adverse inflammatory environment; it may be deleterious to administered cells immediately following reperfusion. Bone marrow mesenchymal stem cells (BMMSCs) have shown great promise in injured myocardium repair.²⁹ However, few studies have explored the potential of BMMSC transplantation for dilated cardiomyopathy (DCM). Yuming and associates³⁰ noted that there is improvement in cardiac function after BMMSC transplantation in rabbits with ischemic type of dilated cardiomyopathy. After four weeks, deposition of collagen fibers in the myocardium of transplantation group was reduced, accompanied by increased expression of VEGF and its receptors as detected by RT-PCR. BMMSC transplantation could alleviate DCM through angiogenesis via the upregulation of VEGF and its receptors.

Several investigators noted that the intense inflammatory reaction after myocardial infarction may cause a local accumulation of mast cells, which might also contribute to the homing of MSCs.³¹⁻³² Others have noted that tissue injury may cause express receptors or ligands expression that facilitate the trafficking and adhesion of stem cells to the injury site, where differentiation is initiated, resulting in the generation of cells of the appropriate lineage.³³⁻³⁵

Wenhui and co-workers noted that intravenously delivered MSCs are capable of time-dependent homing toward the ischemic myocardium even after a delay, promoting regeneration of cardiomyocytes and vessels in vivo. Accordingly, adult stem cells may not only act

locally but may also be recruited from the circulation and be enlisted in the regeneration of diverse tissues at distal sites. Better understanding of the signaling mechanisms that attract bone marrow cells to the ischemic heart and promote differentiation may enhance the therapeutic potential of MSCs in tissue repair.³⁶

Circulating stem cell populations, peripheral levels of hematopoietic growth factors and other cytokines, and the expression of homing factors in the myocardium occur in stem cell biology of heart failure.³⁷ CD34 is a sialomucin expressed by cells with hemangiopoietic potential and a surrogate marker of stem cell potential. CD34 expression by MSCs correlates with enhanced vasculogenic and angiogenic potential.³⁸ A significantly increased circulating CD34(+) cell populations indicate an upregulation of myocardial homing factors in ICM and a reduced homing of stem cells in DCM.³⁹

In nonischemic dilated cardiomyopathy, it was observed by Zhou et al, 2007, in rabbit model that viable cells identified in myocardium at 2nd week post transplantation, with histologic finding of less apoptosis and local tissue movement around injection site on color flow Doppler instrumentation.⁴⁰

In dilated cardiomyopathy, increased expression of CD 34(+) lead to down regulation of myocardial homing factors in DCM hearts attributed to reduced homing of stem cells. CD34(+) expression by MSCs correlates with enhanced vasculogenic and angiogenic potential.³⁸⁻³⁹

The importance of differentiation between ischemic (ICM) and nonischemic (NICM) cannot be overrated; though both present with similar final phenotype of ventricular dilatation and reduced contractility.⁴¹ Cytokine signaling pathways and immediate-early response gene were overrepresented in ICM whereas in NICM, it displayed deregulation of cytoskeletal transcripts, gene encoding for major histocompatibility complex and antigen processing and presentation pathway pointing to an immunologic process.⁴¹

Cardiomyopathies constitute more than 80% of the dilated or congested type. The critical hemodynamic feature is diminished contractile function.⁴² Proposed mechanism of cardiac regeneration with MSC transplantation is through angiogenesis and attenuation of cardiac re-

modeling promoted by conditioned medium of multipotent stromal cells (MSCs) as Hung et al⁴³ delved the molecular events triggered by MSC-conditioned medium (CdM) examining its effects on cultures of primary human aortic endothelial cells (HAECs).⁴⁴ Myocardial fibrosis of dilated cardiomyopathy was inhibited by decreasing matrix metalloproteinase (MMP) expression in rat models. MSCs transplantation significantly increased myocardial arteriolar density and decreased the collagen volume in diabetic myocardium.⁴⁵ Potapova and colleagues showed paracrine factors effects secreted by human mesenchymal stem cells (hMSCs) on endothelial cell migration, extracellular matrix invasion, proliferation, and survival in vitro.⁴⁶ Mishra (2008) cited the full potential of stem cell therapy using allogeneic cells had been attributed to their interaction with host immune response,⁴⁷ where MSC have immunomodulatory properties that suppressed the proliferation of alloreactive T cells on a dose dependent manner. The release of chemokines, cytokines and growth factors secondary to tissue injury cause of recruitment of bone marrow derived MSCs to injured area explained the paracrine effect.

Robert Deans, Vice President of Regenerative Medicine Cleveland based biotech company, mentioned the twelve different types of cells under investigation for treating cardiovascular disease: skeletal myoblast, bone marrow and blood-derived stem cells that have considerable myogenic and angiogenic potential in vitro with safety and feasibility studies in non randomized, non-placebo-controlled trial.⁴⁸⁻⁴⁹ Preclinical studies likewise showed the benefits outweighed the risk of such therapy.⁵⁰

Several route of administration has been proposed. A case series of intramyocardial injection for dilated cardiomyopathy via left thoracotomy exhibit safety and feasibility of autologous bone marrow stem cells.⁵¹⁻⁵² Intracoronary administration of autologous bone marrow-derived skeletal myoblast/progenitor cells in a critically ill with severe heart failure caused by DCM had also been undertaken as well.⁵³⁻⁵⁴ In preclinical trials presented by Nagaya et al (2005), autologous mesenchymal cellular transplantation via intramyocardial route showed myogenesis and angiogenesis and the reduction of myocardial fibrosis.⁵⁵ Zhou and associates

which used intramyocardial route mesenchymal cells, showed less myocardial injury and myocytes apoptosis.⁵⁶

There were various points that affect conversion of marrow cells to nonhematopoietic cells after in vivo transplantation and includes the nature and timing of the injury; marrow mobilization; the marrow cell type infused; the timing of

cell infusion and the number of cells infused; the cell cycle state of the marrow cells, and other functional alterations in the marrow cells from specific injury; the mode of cell delivery; and possibly the presence of microvesicles from injured tissue. All these factors should take into consideration regarding stem cell plasticity.

Table 1. Echocardiographic parameters of patient A who completed 9 sessions of autologous stromal cell infusions

Date	EF% (M-mode/ simpsons)	SV	CO	LV				RA/RV	LA	PAT/TRJ	MR	TR
				EDV	EDD	ESD	ESV					
09-25-07	22% (28%)	69	6	316.4	7.7	6.9	247.3	4.5/4.3	4.9		Severe	N
*02-01-08	31% (42%)	97	8.1	315	7.8	6.5	218	2.7/3.0	4.8	120	Severe	Mild
*04-15-08	29% (35%)	106	9.4	370	8.2	7.1	264	3/2.5	4.8	104	Mild	N
*08-06-04	32% (12%)	153	12.3	474	7.1	6.8	320	3/2.8	5	96	Severe	N
*10-16-08	28% (28%)	134	10	476	7.8	7	341	3/2.8	4.8	96	Moderate	N
*02-04-09	27% (36%)	92	7.6	339	7.9	6.8	246	2.7/2.4	5	120	Severe	N
*04-23-09	28% (35%)	86	7.5	267	8.2	7.1	181	2.6/2.6	5.2	117/21	Mild	N
*08-20-09	22% (29%)	82	7.3	376	8.3	7.4	293	2.3/2.4	5.6	104	Severe	Mild
*09-23-09	24% (32%)	87		365	8.2	7.2	277	2.7/2.5	5	104	Severe	Mild
*10-03-09	24% (32%)	107	7.1	443	9	7.9	336	3.3/3.3	5.8	120	Moderate	Mild
*03-09-10	20% (27%)	71	6.2	357	8.1	7.3	285	2.6/2.1	5	96	Severe	Mild
*02-24-11	24% (23%)	162	16.5	636	8.6	7.8	474	5.12/4	5.6	54	Severe	Severe
*03-02-11	22% (25%)	95		376	7.8	6.9	282	5/3.3	5.7	30/33	Moderate	N

EF ejection fraction SV stroke volume CO cardiac output LV left ventricle EDV end-diastolic volume EDD end-diastolic diameter ESV end-systolic volume ESD end-systolic diameter RA right atrium RV right ventricle LA left atrium PAT Pulmonary acceleration time TRJ tricuspid regurgitation jet MR mitral regurgitation TR tricuspid regurgitation

Table 2. Echocardiographic parameters of patient B who completed 7 sessions of autologous stromal cell infusion

Date	EF% (M-mode/ simpsons)	SV	CO	LV				RA/RV	LA	PAP	MV		TR
				EDV	ESV	EDD	ESD				E O A (cm ²)	PDPG	
05-05-09	38% (50%)	77.4	7.4	201.2	123.8	6.3	5.1	6.2/4.9	7.2	Mild PAH	2.1	4	Mild
*08-20-09	48% (57%)	98.6	7.2	223.6	125	6.6	5.0	5.7/4.2	6.7	N	2	5	Mild
*09-07-09	41.6% (53%)	70.96	4.97	170.4	99.44	5.9	4.6	6.1/4.3	6.8	N	2	4.2	Severe
*11-26-09	57% (65%)	127.5	8.8	231.3	103.8	6.7	4.7	5.0/4.1	7.0	N	2	5	Mild
*01-08-10	56% (66%)	84.7	6.5	135.3	50.6	5.3	3.7	4.8/4.0	7.3	N	2	5	Mild
*03-12-10	51% (60%)	123.8	10.1	247.3	123.8	6.9	5.1	4.0/4.0	8.0	N	2	5	Mild
*05-27-10	35% (44%)	70.2	5.6	194	123.8	6.2	5.1	5.4/5.8	7.3	Mild PAH	2	3	Moderate

EF ejection fraction SV stroke volume CO cardiac output LV left ventricle EDV end-diastolic volume EDD end-diastolic diameter ESV end-systolic volume ESD end-systolic diameter RA right atrium RV right ventricle LA left atrium PAT Pulmonary acceleration time TRJ tricuspid regurgitation jet MV mitral valve TR tricuspid regurgitation EOA-effective orifice area PDPG-peak diastolic pressure gradient *Post in fusion results of 2d echocardiography

	PATIENT A		PATIENT B (s/p MVR)	
	Pre (09-22-07)	Post (09-23-09)	Pre (05-05--09)	Post (11-26-09)
Parasternal Long axis				
Short Axis Left Ventricle Mitral valve (basal-level)				
Papillary muscles (mid-level)				
Left Ventricular Apex				
Apical Four Chamber				
LVEDD	7.7 cm	8.2 cm	6.3 cm	6.7 cm
LVESV	6.9 cm	7.2 cm	5.1 cm	4.7 cm
EF	22%	24%	38%	57%
CO	6 L/min	8.1 L/min	7.4 L/min	8.8 L/min
SV	69.1 ml	87 ml	77.4.1 ml	127.5 ml

Figure 1. Echocardiographic parameters pre and post stem cell infusion of two patients who had dilated cardiomyopathy

CONCLUSION

With regards to the type of stem cells given, we used purified bone marrow cells. The treatment received by our patients was designed utilizing purified mesenchymal stem cells to eradicate other cell types that may be detrimental to a patient with severe compromise myocardial conditions as the effect of thrombocytes causes clot formation.

In conclusion, both of our patients responded well to stem cell infusion. As shown in echocardiographic parameters (Figure 1) such as left ventricular end systolic volume, left ventricular end diastolic volume, stroke volume, cardiac output and ejection fraction. Both of them exhibited highest ejection fraction after the 3rd infusion. One noticeable response was a decline in ejection fraction after infectious process. The first patient survived 4 years from the time of infusion and currently is still employed as a police officer. Our second patient had 9 months extension since infusion. Mortality secondary to complication of an abdominal infection that resulted to sepsis lead to development of seizure and subsequent multi-organ failure. Both of them were able to work after every infusion.

The widening of QRS complexes noted during the first infusion was not noted in subsequent infusion. This was probably due to the initial reaction to the reagent in conditioned media. It had been shown a certain gene Ser96Ala is associated with life-threatening ventricular arrhythmias in idiopathic DCM and may serve as an independent predictor of susceptibility to arrhythmogenesis in the setting of DCM. However, study by Tigen K et al 2008,⁵⁷ showed electrocardiographic parameters failed to predict clinical end points in patients with non-ischemic dilated cardiomyopathy, instead Plasma NT pro-BNP maybe useful biochemical marker to define the high-risk group that warrants closer follow-up in dilated cardiomyopathy patients with sinus rhythm. Our second patient cardiac rhythm was atrial fibrillation with controlled ventricular response, therefore outcome evaluation was based mostly on parameter on 2D echocardiography.

Our first patient experienced generalized warmth sensation after every infusion, which maybe a natural reaction to cell components.

Breast tenderness was attributed to granulocyte colony stimulating factor that was added to the cell preparation. The second patient showed exact opposite effect of generalized cold chilly sensation. This could be attributed to drug-drug interaction of his various maintenance medications, one of which is warfarin for his mechanical prosthetic valve.

The overall outcome of purified bone marrow derived mesenchymal cell therapy demonstrated an initial improvement in ejection fraction on echocardiography. The homing mechanism and improvement in cardiac function is manifested by a remarkable contractility seen on the right ventricular function.

MET scoring indicated for cardiac rehabilitation prescription of activities was used as a gauge for activities of daily living and quality of life assessment. Absence of symptoms and resumption of employment suggests good quality of life with favorable outcome of this therapy.

Various factors may affect the success of cell therapy: specific type of stem cells from a specific source; amount of cells to be utilized; appropriate mode of administration and others external factors. This is a pioneering approach of intravenously administered autologous bone marrow derived mesenchymal stromal stem cells utilizing minimally invasive technique through slow infusion. This technique reduces endothelial injury and arrhythmogenicity where the overall observation of this study suggest safety and feasibility, along with our clinical outcome leads us to convey this alternative therapy has a role in cardiac regeneration of dilated cardiomyopathy non ischemic type. This report may serve as a guide for further search of this new individualized therapeutic strategy of treatment.

Ethical Considerations

Written informed consent was obtained from the patients for the procedure and its publication as well as the diagnostic images.

COMPETING INTEREST

The authors declare no competing interest.

AUTHOR'S CONTRIBUTIONS

Dr. Ruth Ong collected the data and drafted the

manuscript. Dr. Avenilo P. Aventura, Dr. Samuel Bernal, Dr. Santos-Abad Jose, Dr. Teresita de Guia corrected and revised the manuscript. All authors have read and approved the final manuscript.

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