

Autologous Concentrate Bone Marrow Cell Therapy for Ischemic Cardiomyopathy Unsuited for Revascularization: Feasibility Study

Abstract

Stem Cells, platelets, and cytokines cooperate to repair ischemic myocardium. Autologous bone marrow aspirate concentrate (BMAC), obtained using a point of care device, has all the component in their natural plasma environment.

Objectives: To assess the feasibility and safety to harvest and implant BMAC, in the same session, into myocardium via left anterior thoracotomy, in patients with untreatable ischemic cardiomyopathy. **Methods:** presence of viable myocardium was assessed with dobutamine echocardiography. Segments ischemic/hibernated were: apical anterior, medial anterior, apical lateral, medial, basal lateral. Bone marrow harvested from iliac crest and centrifuged with a point of care device, was implanted through left anterior thoracotomy into hibernated myocardium (20 injections, 1 ml each). Segments treated were: medial anterior (10 ml), medial and basal lateral (10 ml).

Results: five patients with untreatable coronary artery disease (CAD) underwent BMAC implantation; four patients had ischemic congestive heart failure, one had Canadian class III angina. All patients completed one-year follow-up; three patients had history of AMI, 2 had coronary revascularization; 5 patients were in New York Heart Association functional class III/IV; all pts had a mean of 3 hospital admission; at follow-up they were in class I/II. EF% improved by 14%, 19% and 30% at 3, 6, 12 months follow-up ($p = 0.008$). Variation of cardiac echocardiographic parameters, demonstrate similar improvement.

Conclusion: In 5 patients with ischemic cardiomyopathy treated with BMAC, EF, NYHA class and quality of life are improved. Further studies are necessary to confirm these results.

Keywords: Stem cells; Bone marrow; Ischemic cardiomyopathy; Angina

Research Article

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Eugenio Caradonna^{1*}, Carlo Maria De Filippo¹, Nicola Testa¹, Giovanna Di Giannuario², Mariangela Amatuzio¹, Saki Bogias¹, Stefano Papini¹, LiciaIacoviello³, Simona Costanzo³, Massimo Massetti⁴ and David B Harrell⁵

¹Fondazione di Ricerca e Cura Giovanni Paolo II, Università Cattolica del Sacro Cuore, Italy

²Ospedale San Raffaele, Italy

³Laboratory of Molecular and Nutritional Epidemiology, Department of Epidemiology and Prevention, IRCCS Istituto Neurologico Mediterraneo Neuromed, Italy

⁴Department of Cardiac Surgery, Policlinico Gemelli, Università Cattolica del Sacro Cuore, Italy

⁵Harrell BioScience Consulting, USA

***Corresponding author:** Eugenio Caradonna, Fondazione di Ricerca e Cura Giovanni Paolo II, Università Cattolica del Sacro Cuore, Largo Gemelli 1, 86100 Campobasso, Italy, Tel: 390874312320; Email: eugenio.caradonna@gmail.com

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Abbreviations: BMSCs: Bone Marrow Stem Cells; CSCs: Cardiac Stem Cells; VEGF: Vascular Endothelial Growth Factor; BMAC: Bone Marrow Aspirate Concentrate; PBS: Phosphate-Buffered Saline; BMA: Bone Marrow Aspirate; EDD: End Diastolic Diameter; EDV 2C: End Diastolic Volume 2 Chamber; EDV 4C: End Diastolic Volume 4 Chamber; ESD: End Systolic Diameter; ESV 2C: End Systolic Volume 2 Chamber; ESV 4C: End Systolic Volume 4 Chamber

Introduction

The incidence of refractory angina and ischemic cardiomyopathy is increasing and novel therapeutic options are necessary [1]. Implantation of bone marrow stem cells (BMSCs) in the myocardium is feasible and safe [2,3]. However, is still unclear which is the best therapeutic strategy. Isolated BMSCs, mainly CD34+ CD133+, have been used in almost all published studies. Recent scientific evidence suggest the importance of cooperation between different type of cells, CD133+, CD34+, c-kit, cardiac stem cells (CSCs), platelets and cytokines, to repair the ischemic myocardium [4-9]. The process to repair the ischemic

myocardium is complex and involves CSCs, BMSCs, cytokines, and platelets [10]. For example, hibernated myocardium is characterized by an increased expression of cytokines, such as vascular endothelial growth factor (VEGF), that appear to be important for both protection and functional recovery [11,12]. Autologous bone marrow aspirate concentrate (BMAC) obtained by using a point of care device, consists of a heterogeneous cell population including hematopoietic and mesenchymal stem/progenitor cells as well as granulocytes and platelets. BMAC has the full complement of the nucleated cellular niche suspended in its natural plasma environment. Platelets and cytokines (e.g., VEGF, stromal derived factor-1 α , transforming growth factor- β 1, platelets derived growth factor) are present [13]. Stem cells are not manipulated. Their homing properties are not impaired as when obtained with culture and density gradient methods [14-17]. BMAC has been infused intracoronary in patients with positive results [18]. To assess the feasibility, safety and efficacy of BMAC implantation, we treated five patients with ischemic congestive heart failure (4 pts.) and Canadian class III angina (1 pt.) with BMAC implantation directly into the myocardium via a left anterior thoracotomy.

Materials and Methods

As a feasibility study, approved by The Catholic University Ethical Committee, five patients with ischemic congestive heart failure (4 pts.) and Canadian class III angina (1 pt.) underwent autologous fresh BMAC implantation via left anterior thoracotomy. Our internal heart team, as well as by other independent institutions, considered inoperable all patients due to the poor quality of the coronary arteries. All patients gave written informed consent. We have chosen dobutamine echocardiography (10 μ /kg/ min.) to evaluate the presence of viable myocardium due to the fact that this category of patient has often contraindication to magnetic resonance imaging (ICD, resynchronization device). The cardiac segments were divided using the sixteen model of the European Association of Echocardiography. The segments ischemic/hibernated were: apical anterior, medial anterior, apical lateral, as well as medial and basal lateral.

Peripheral blood samples for each patient were sent to the laboratory for cytometric analysis the day before implantation and the first postoperative day. Mesenchymal cells phenotyping was performed by both immuno staining and flow cytometry using antibody-fluorochrome conjugates for 30 minutes on ice (BECKMAN COULTER NAVIOS, Via Roma, 108 - Palazzo F1Centro Cassina Plaza20060 - Cassina De' Pecchi, Milan, Italy). Appropriate isotype controls were also used (BECKMAN COULTER). Stained cells were washed, suspended in phosphate-buffered saline (PBS) and analyzed using a low cytometer (BECKMAN COULTER-NAVIOS ISHAGE). Standard Protocols were used for enumerating CD34+ and CD133+ cells. The flow cytometry data were analyzed with an appropriate software (BECKMAN COULTER).

Surgical technique

Under general anesthesia, 120 ml of bone marrow aspirate (BMA), harvested from the posterior iliac crest were processed to produce 20 ml of BMAC using a point-of-care system (Harvest BMAC System; Harvest Technologies GmbH, Munich, Germany) according to the manufacturer's instructions. One ml of BMA and 1 ml of BMAC were sent to the laboratory for cytometric analysis. During the 15 minutes processing period, the patient was repositioned to a supine position. Through a small left anterior thoracotomy, BMAC was implanted into the myocardium via 20 injections of 1 ml each (22 gauge needle). The segments treated, according to the preoperative data, were: medial anterior (10 ml), as well as medial and basal lateral (10 ml).

Perioperative care

Cardiac index was measured on an hourly base. Continuous electrocardiographic monitoring with automatic arrhythmia detection was maintained for 7 days' post surgery. Echocardiography was performed inter-operatively and at one week. Follow-up visits were conducted at 3, 6 and 12 months. During follow-up all patients underwent 24-hour Holter recording, and transthoracic echocardiography. All patients completed one-year follow-up. Cardiac echocardiographic parameters measured were: EF %, End Diastolic Diameter (EDD), End Diastolic Volume 2 chamber (EDV 2C), End Diastolic Volume 4 chamber (EDV 4C),

End Systolic Diameter (ESD), End Systolic Volume 2 chamber (ESV 2C), End Systolic Volume 4 chamber (ESV 4C).

The post operative echocardiographic exams were performed by the same person (***) not aware of the treatment.

Statistical analysis

The serial time measurements have been evaluated by Friedman's test (non parametric test for repeated measures). The pair wise comparison of the relative change of the echocardiographic parameters during follow-up (3, 6 and 12 months) with baseline measurements obtained before BMAC administration was performed by the non-parametric Wilcoxon Signed Rank test, adjusted for multiple comparisons by Bonferroni's test. The relative change of the Echocardiographic Parameters for each patient, was measured as $100 \times (Y_{\text{follow-up}} - Y_{\text{baseline}}) / Y_{\text{baseline}}$, where Y is one of the echocardiographic Parameters.

Data are reported as means and standard error of the mean (SEM); p-values below 0.05 were considered as statistically significant. All computations were carried out using SAS statistical software (Version 9.1.3 of the SAS System for Windows 2009. SAS Institute Inc. Cary, NC, USA).

Results

Preoperative baseline and postoperative characteristics of the 5 recruited patients are summarized in Table 1. The average age of 5 patients was 67.6 years ($\pm 3, 5$), only one was female. Three patients had a history of previous AMI and 2 were underwent previous coronary revascularization. All pts had a mean of 3 hospital admission. Pre-surgery, all patients were in New York Heart Association functional class III/IV and after BMAC implantation they shifted to New York Heart Association functional class I/II. Tables 2 and 3 show the panel of cells present in BMA and BMAC, as well as variation of stem cell one day before and one day after the surgery (Table 3). The first day after BMAC implantation the number of CD34+ and CD133+ increased by 140% (± 30) and 250% (± 45) (p=0.06, Table 3 B). The mean value of ejection fraction (EF) improved over time (p = 0.002). EF measurements improved by 14%, 19% and 30% at 3, 6 and 12 months (p = 0.008). Figure 1A and Table 4 show the relative change of EF at 3, 6 and 12 months, compared with the baseline measurement. During follow-up the mean values of end systolic and end diastolic volumes decreased (Table 4, p 0.05) The relative change of other Cardiac Echocardiographic Parameters (ESV 4C, ESV 2C, EDV 4C and EDV 2C) during follow-up, demonstrate similar improvement (Figure 1B and Table 4, p< 0.05).

During the hospital stay no arrhythmias occurred. The postoperative course was uneventful and all patients were discharged on the 7th postoperative day. The patient for whom the indication was Canadian class III angina is in Canadian Class I. The patients who had icd and history of ventricular arrhythmias before BMAC implantation, had one admission to the hospital for recurrent ventricular arrhythmias. All the other four pts had no hospital admission.

Table 1: Baseline characteristics of 5 patients with ischemic cardiomyopathy and autologous fresh BMAC implantation.

AMI: Acute Myocardial Infarction; COPD: Chronic Obstructive Pulmonary Disease; CVD: Cardiovascular Disease; SEM: Standard Error of the Mean.

Data presented as No (%) unless otherwise specified.

New York Heart Association class I indicates no limitation; class II, slight limitation of physical activity; and class III, marked limitation of physical activity. Ex-smoker indicates patient was abstinent from cigarettes for at least one year before enrolment.

Baseline Characteristics	N (%)
Age, mean (SEM)	67.6 (3.5)
Male sex	4 (80)
CVD Family history	2 (40)
History of AMI	3 (60)
Carotid artery atheroma	3 (60)
Implantable cardioverter-defibrillator	1 (20)
Peripheral arterial disease	3 (60)
Intermittent claudication	3 (60)
History of previous coronary revascularization	2 (40)
History of atrial or ventricular arrhythmia	1 (20)
History of hypertension	4 (80)
History of diabetes mellitus	4 (80)
History of hyperlipidemia	5 (100)
Renal failure	1 (20)
Pre-surgery New York Heart Association class	
I	0
II	0
III	3 (60)
IV	2 (40)
Post-surgery New York Heart Association class	
I	4 (80)
II	1 (20)
III	0
IV	0
History of smoking	4 (80)
Ex-smoker	4 (80)
Sigarette/day, mean (SEM)	18.75 (1.25)

Table 2: Panel of cells of bone marrow, BMAC and peripheral stem cell the day before implant and the first post-operative day in 5 patients with ischemic cardiomyopathy.

Patients	Platelets	CD34+	CD34+	CD133+	CD133+	CD117+	CD117+
	10 ³ x mm ³	%	cell x 10 ⁶ ml	%	cell x 10 ⁶ ml	%	cell x 10 ⁶ ml
Pt. 1							
1 Day Pre-Operative	99	1.78%	0.25	0.15%	0.021	0.96%	0.14
1 Day Post-Operative	401	1.97%	1.31	0.16%	0.104	1.29%	0.85
Pt. 2							
1 Day Pre-Operative	109	0.66%	0.05	0.10%	0.008	0.38%	0.03
1 Day Post-Operative	641	0.88%	0.36	0.04%	0.018	0.50%	0.2
Pt. 3							
1 Day Pre-Operative	118	1.02%	0.17	0.23%	0.038	0.15%	0.025
1 Day Post-Operative	390	1.19%	0.65	0.30%	0.16	0.18%	0.099
Pt. 4							
1 Day Pre-Operative	121	2.05%	0.69	0.12%	0.08	2.68%	0.81
1 Day Post-Operative	43	2.30%	0.99	0.13%	0.11	2.61%	1.4
Pt. 5							
1 Day Pre-Operative	112	1.13%	0.22	0.13%	0.02	1.05%	0.2
1 Day Post-Operative	487	1.02%	0.85	0.10%	0.08	0.92%	0.77

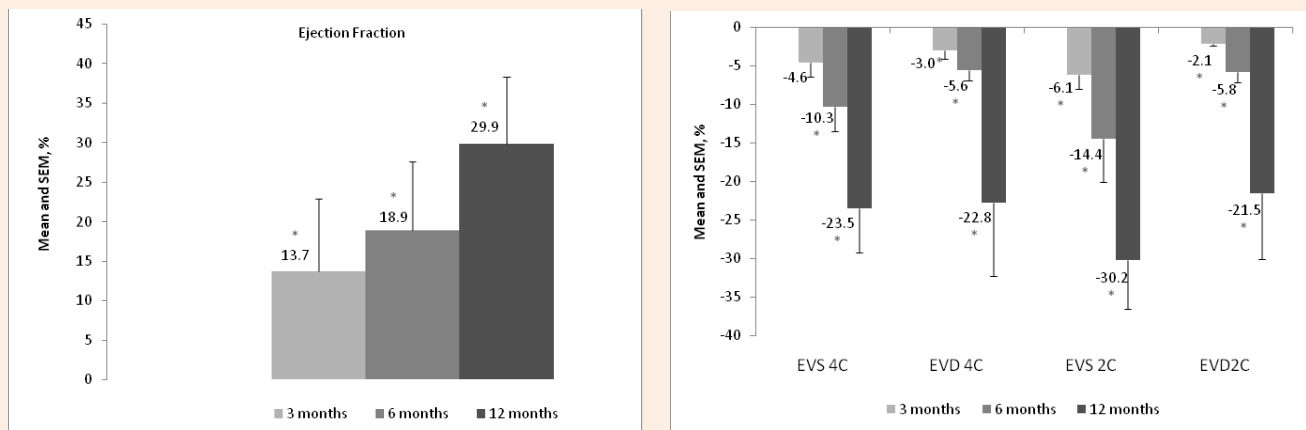


Figure 1: Relative change of Ejection Fraction (A) and Echocardiographic Parameters (B) at 3, 6 and 12 months in 5 patients with ischemic cardiomyopathy underwent autologous fresh BMAC implantation.

EFX: Ejection Fraction at 3, 6 or 12 months; EF0: Ejection Fraction at baseline.

YX: Echocardiographic Parameters at 3, 6 or 12 months; Y0: Echocardiographic Parameters at baseline; EDD: End Diastolic Diameter; EDV 2C: End Diastolic Volume 2 Chamber; EDV 4C: End Diastolic Volume 4 Chamber; ESD: End Systolic Diameter; ESV 2C: End Systolic Volume 2 Chamber; ESV 4C: End Systolic Volume 4 Chamber.

*P= 0.06 Wilcoxon Signed Rank Test (reference baseline measurement); P= 0.18 adjusted by Bonferroni's Test.

Table 3: Mean and SEM of the amount of Peripheral Stem Cells, the day before implant and the first post-operative day, in 5 patients with ischemic cardiomyopathy underwent autologous fresh BMAC implantation.

Abbreviations: $\Delta = ((\text{Post-surgery} - \text{Pre-surgery}) / \text{Pre-surgery}) \times 100$

*Wilcoxon Signed Rank Test.

	1 Day Pre-Surgery	1 Day Post-Surgery	Δ , %	p value*
CD34+, cell x 10 ⁶ ml	0.0064 (0.68 10 ⁻³)	0.0148 (1.28·10 ⁻³)	140 (30)	0.0625
CD133+, cell x 10 ⁶ ml	0.0016 (0.6 x10 ⁻³)	0.0062 (2.97·10 ⁻³)	250 (45)	0.0625

Table 4: Mean absolute and relative changes of the Cardiac Echocardiographic Parameters Pre-operatively and at 3, 6 and 12 months in 5 patients with ischemic cardiomyopathy which underwent autologous fresh BMAC implantation.

EF: Ejection Fraction; EDD: End Diastolic Diameter; EDV 2C: End Diastolic Volume 2 Chamber; EDV 4C: End Diastolic Volume 4 Chamber; ESD: End Systolic Diameter; ESV 2C: End Systolic Volume 2 Chamber; ESV 4C: End Systolic Volume 4 Chamber.

*Friedman's test for repeated measures.

Cardiac Echocardiographic Parameters	Baseline	3 Months	6 Months	12 Months	p value*
Time, Mean (SEM), months	-	2.9 (0.3)	5.7 (0.4)	11.8 (0.8)	-
ESD					
Mean (SEM), mm	41 (4.0)	39.6 (3.2)	39.2 (3.2)	42.2 (3.8)	0.414
Absolute change, mm		-1.4 (1.0)	-1.8 (1.1)	1.2 (3.5)	0.646
Relative change, %		-2.7 (2.1)	-3.8 (2.3)	4.9 (10.6)	0.646
EDD					
Mean (SEM), mm	50.4 (5.1)	49.4 (4.7)	49.2 (4.9)	52.4 (3.8)	0.284
Absolute change, mm	-	-1.0 (0.5)	-1.2 (1.0)	2.0 (5.2)	0.486
Relative change, %		-1.7 (0.8)	-2.0(2.2)	8.2 (14.4)	0.486
ESV 4C					
Mean (SEM), mL	108.4 (27.5)	104.6 (27.2)	99 (27.3)	78.4 (17.3)	0.004
Absolute change, mL		-3.8 (1.4)	-9.4 (2.6)	-30.0 (11.6)	0.022
Relative change, %		-4.6 (1.8)	-10.3 (3.2)	-23.5(5.7)	0.022
EDV 4C					
Mean (SEM), mL	178.8 (47.3)	173.2 (45.5)	168.0 (43.6)	121.0 (18.4)	0.002
Absolute change, mL		-5.6 (2.4)	-10.8 (4.9)	-57.8 (31.2)	0.008
Relative change, %		-3.0 (1.1)	-5.6 (1.3)	-22.8 (9.5)	0.008
ESV 2C					
Mean (SEM), mL	110.8 (28.0)	104.0 (26.8)	97.4 (28.4)	72.0 (15.0)	0.002
Absolute change, mL		-6.8 (2.6)	-13.4 (5.4)	-38.8 (14.5)	0.008
Relative change, %		-6.1 (1.9)	-14.4 (5.7)	-30.2(6.3)	0.008
EDV 2C					
Mean (SEM), mL	175.4 (46.5)	171.4 (45.1)	165.2 (43.7)	122.6 (19.6)	0.002
Absolute change, mL		-4.0 (1.4)	-10.2 (4.1)	-52.8 (27.9)	0.008
Relative change, %		-2.1 (0.3)	-5.8(1.3)	-21.5 (8.5)	0.008
EF					
Mean (SEM), %	35.8 (5.0)	40.0 (4.8)	42.0 (5.2)	45.4 (4.5)	0.002
Absolute change, %	-	4.2 (2.7)	6.2 (2.5)	9.6 (2.2)	0.008
Relative change, %		13.7 (9.1)	18.9 (8.7)	29.9 (8.4)	0.008

Discussion

BMSCs have been used in ischemic heart failure with limited positive results and conflicting evidence [2, 3]. A single population of cells was delivered in almost all previous studies. Recent research demonstrates that the use of complementary cells with synergistic action may enhance therapeutic outcomes [4-9]. The interaction between different cells is regulated by paracrine mediators that are important for neoangiogenesis and positive cardiac remodelling [9]. BMAC yields an high concentration of cytokines (VEGF, SDF-1 α , TGF- β 1, PDGF) [13]. Cytokines (e.g., VEGF, SDF-1 α , PDGF) are triggered by the ischemic events and play a major role in the interactions between cells, during the process to restore the damaged myocardium and in the evolution of hibernation toward apoptosis [19]. VEGF promote angiogenesis, improves cardiac function and is involved in the repair of the ischemic myocardium [20-23]. SDF-1 α has inotropic action [25]. Platelets produce several factors as VEGF, SDF-1, that are important for homing, differentiation of endothelial progenitor cell toward endothelial cell and mobilization of cardiac stem cells for myocardial repair [24,25]. Platelets promote angiogenesis and differentiation of CD34+ in endothelial cells [23,26]. Bone marrow stem cells have an important role in the process to repair and protect the ischemic myocardium [27]. C-kit cells are cardioprotective and regulate angiogenesis [28]; C117 (c-kit) are present in BMAC (Table 2). Hibernated myocardium is characterized by an increased expression of cytokines; however stem cells obtained by culture and density gradient centrifugation express a reduced number of active receptors for cytokines as SDF-1 [16,17]. Turan et al. [18] observed a statistically significant increase in the number of circulating CD34+ and CD133+ the day after intracoronary injection of BMAC [18]. We found a similar increase the day after intramyocardial injection (Table 2). This increase could be due to poor homing of the cells or to the presence of the cytokines in the BMAC. The number and viability of cells obtained with a point of care device compare positively with the standard Ficoll method [13]. Density gradient method centrifugation alter the cellular yield [14]. Homing is impaired in cell cultured or obtained by density gradient centrifugation [15,14,29]. Stem cells obtained with a point of care device are not manipulated. Williams et al. [30] reported 8 patients with ischemic cardiomyopathy previous myocardial infarction treated with transendocardial injections of mesenchymal stem cell (4 patients) after culture and bone marrow mononucleated cell (4 patients) obtained by density gradient centrifugation (Ficoll method) [30]. There was a positive trend toward improvement in the left ventricle volume and dimension; however, the EF and ventricular mass did not change [31]. Our small series demonstrated a significant improvement in the dimension and volume of the left ventricle and in the EF. In a recent review the mean increase in EF was 3.96%). In our patients, EF% improved by 14%, 19% and 30% at 3, 6 and 12 months of the follow-up, respectively (p = 0.008).

BMAC contains the majority of the components for repair of the ischemic damage and has a particularly elevated level of VEGF and SDF-1 α [13]. Injection of intracoronary stem cells has inherent limitation related to homing and the possibility of micro infarctions [32]. The endomyocardial route does not allow accurate delivery [33].

A minimally invasive left anterior thoracotomy (3-5 cm) allows injecting BMAC precisely into the target segment. The operation (bone marrow aspiration, centrifugation and injection) lasts less than two hours and appears to be safe with minimal discomfort for the patient. The amount of BM harvested (120 ml) is under the volume that could cause anaemia and blood volume pooling. In this feasibility study, patients with ischemic cardiomyopathy and inoperable CAD treated with BMAC, have had a substantial improvement in EF, NYHA class, left ventricle positive remodelling, and in the quality of life (Table 1 and Figure 1). BMAC has the following advantages:

- a) Stem cells are not manipulated,
- b) Bone marrow can be harvested and implanted in the same operative session,
- c) The procedure is cost effective.

As such, BMAC implantation could be useful in selected cases. However, given that our study only incorporated five patients further studies with larger numbers of patients are needed to confirm these results. The design and the results of this study are the fundament of a multicenter randomized trial for ischemic cardiomyopathy unsuitable for revascularization.

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