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# The Role of PET with $^{13}\text{N}$ -Ammonia and $^{18}\text{F}$ -FDG in the Assessment of Myocardial Perfusion and Metabolism in Patients with Recent AMI and Intracoronary Stem Cell Injection

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Over the last decade, the effects of stem cell therapy on cardiac repair after acute myocardial infarction (AMI) have been investigated with different imaging techniques. We evaluated a new imaging approach using  $^{13}\text{N}$ -ammonia and  $^{18}\text{F}$ -FDG PET for a combined analysis of cardiac perfusion, metabolism, and function in patients treated with intracoronary injection of endothelial progenitors or with conventional therapy for AMI. **Methods:** A total of 15 patients were randomly assigned to 3 groups based on different treatments (group A: bone marrow-derived stem cells; group B: peripheral blood-derived stem cells; group C: standard therapy alone). The number of scarred and viable segments, along with the infarct size and the extent of the viable area, were determined on a 9-segment  $^{13}\text{N}$ -ammonia/ $^{18}\text{F}$ -FDG PET polar map. Myocardial blood flow (MBF) was calculated for each segment on the ammonia polar map, whereas a global evaluation of left ventricular function was obtained by estimating left ventricular ejection fraction (LVEF) and end-diastolic volume, both derived from electrocardiography-gated  $^{18}\text{F}$ -FDG images. Both intragroup and intergroup comparative analyses of the mean values of each parameter were performed at baseline and 3, 6, and 12 mo after AMI. During follow-up, major cardiac events were also registered. **Results:** A significant decrease ( $P < 0.05$ ) in the number of scarred segments and infarct size was observed in group A, along with an increase in MBF ( $P < 0.05$ ) and a mild improvement in cardiac function. Lack of infarct size shrinkage in group B was associated with a marked impairment of MBF ( $P = 0.01$ ) and cardiac dysfunction. Ambiguous changes in infarct size, MBF, and LVEF were found in group C. No differences in number of viable segments or in extent of viable area were found among the groups. At clinical follow-up, no major cardiac events occurred in group A patients, whereas 2 patients of group B experienced in-stent occlusion and one patient of group C received a transplant for heart failure. **Conclusion:** Our data suggest that a single nuclear imaging technique accurately analyzes changes in myocardial perfusion and metabolism occurring after stem cell transplantation.

**Key Words:** acute myocardial infarction; stem cells; PET; ammonia; FDG-gated

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**D**espite progress made in the treatment of acute myocardial infarction (AMI) based on early reperfusion of the culprit artery, the lack of repair of a great amount of damaged cardiac tissue may lead to congestive heart failure, whose medical and social implications justify the use of experimental approaches, especially in high-risk patients (1). The use of stem cells with angiogenic properties for therapy of myocardial infarction has been widely investigated, and several clinical studies have demonstrated the feasibility of intracoronary injection of various cell preparations derived from bone marrow and peripheral blood (2–8). Nevertheless, the efficacy of this novel treatment is still under investigation, it being unclear whether patients who have undergone stem cell therapy have a greater improvement in left ventricular function (LVEF) and a greater reduction of infarct size than do patients treated with standard therapy for AMI (3,4,9–17). Besides the different imaging methods used for the estimation of LVEF and infarct size, a quantitative evaluation of myocardial blood flow (MBF) in scarred and non-infarct-related areas has been attempted only with  $^{11}\text{C}$ -acetate PET (8). Because of its ability to provide a noninvasive absolute quantification of MBF (18–19),  $^{13}\text{N}$ -ammonia PET has been widely used to assess microvascular dysfunction in asymptomatic subjects with cardiovascular risk factors (20) and in patients with coronary artery disease and other cardiac disorders (21,22).

In the course of a study designed to evaluate the safety and feasibility of intracoronary injection of endothelial progenitors after AMI and unsuccessful primary angioplasty, we first aimed to quantify cardiac perfusion in 2 groups of patients treated with stem cells derived from bone

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marrow or mobilized in peripheral blood and in 1 group with a standard therapy for infarction. The contemporary assessment of the changes in metabolism and in left ventricular function by means of a single  $^{18}\text{F}$ -FDG PET gated acquisition was another attractive endpoint of our study.

## MATERIALS AND METHODS

### Patients

We enrolled 15 patients (14 men and 1 woman; age range, 30–64 y) with recent ST-elevation myocardial infarction due to thrombotic occlusion of the left anterior descending artery and percutaneous intervention with bare metal stent implantation within 6 h from symptom onset. The inclusion criteria were age between 18 and 65 y, single-vessel disease, anterior infarction with more than 2-mm ST-segment elevation in all 6 electrocardiography chest leads, and successful recanalization (thrombolysis in myocardial infarction grade 2–3 flow) with impaired reperfusion (myocardial blush 0 or 1 at the end of the procedure and ST-segment recovery less than 50% 1 h after percutaneous intervention) (23,24). The exclusion criteria were previous myocardial infarction, an indication for aortocoronary bypass grafting, primary bone marrow diseases, diabetes, immunosuppressive therapy, congenital coagulation protein disorders, or severe comorbidity. The clinical characteristics, infarct-related cardiac enzyme levels, and angiographic pattern of the patients are summarized in Supplemental Table 1.

### Study Design

After giving informed consent, the eligible patients were randomly assigned to 3 different therapy groups. Group A received an intracoronary injection of CD133+ cells after bone marrow harvesting under general anesthesia. Group B had an intracoronary injection of purified CD133+ cells after a 4-d course of granulocyte colony-stimulating factor (5  $\mu\text{g}/\text{kg}/\text{d}$ ) followed by leukapheresis. The patients randomized to control group C were given standard medical therapy alone. All patients underwent baseline  $^{13}\text{N}$ -ammonia/ $^{18}\text{F}$ -FDG gated PET between 6 and 11 d after AMI, to evaluate myocardial perfusion, metabolism, and contractile function of the left ventricle, the last of these also being assessed with echocardiography. All patients underwent clinical and instrumental follow-up. PET studies and cardiac ultrasonography were repeated at 3, 6, and 12 mo.

The study protocol conformed with the Declaration of Helsinki and was approved by the local Ethics Committee of the 3 institutions involved (Fondazione IRCCS Ca' Granda, Luigi Sacco Hospital, and Fondazione IRCCS San Donato) and by the National Committee responsible for authorizing phase I cell therapy protocols (Istituto Superiore di Sanità). An independent Data and Safety Monitoring Committee was informed of the adverse events that occurred during the study.

### Cell Preparation and Administration

The cells were prepared according to good manufacturing practices with a closed, automated system (CliniMACS; Miltenyi Biotec) used for the immunomagnetic selection of CD133+ cells. Bone marrow-derived cells were processed immediately after harvesting; peripheral blood-derived cells were stored overnight at  $4^\circ\text{C} \pm 2^\circ\text{C}$  and processed within 24 h of collection. The final cell product underwent quality controls, including sterility tests for aerobic and anaerobic bacteria and fungi, cell count, viability tests, and flow cytometry analysis. The expression of CD133+ was evaluated on the purified cells using the clone AC133/2

(CD133-PE antibody; Miltenyi Biotec). The median number of injected bone marrow and peripheral blood-derived cells was  $5.9 \times 10^6$  (range,  $4.9 \pm 13.5$ ) and  $68.3 \times 10^6$  (range,  $16.0 \pm 355.9$ ), respectively. The cells were infused within 3 h of preparation, between the 11th and the 15th day after AMI.

After stenting of the infarct-related coronary artery, an over-the-wire angioplasty balloon was positioned inside the lumen of the stent and was inflated at low pressure to occlude the vessel. Then, the guidewire was removed and the cells were infused into the lumen of the balloon during a 3-min-long vessel occlusion, according to the methodology proposed by Strauer et al. (2). This stop-flow technique was deemed appropriate to limit dilution of the cells and to promote their distribution to the surrounding tissue.

All patients had been placed on angiotensin-converting-enzyme inhibitors,  $\beta$ -blockers, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors in addition to aspirin and clopidogrel since admission. Clopidogrel was withdrawn 1 mo after AMI. All patients continued their medical therapy throughout the study.

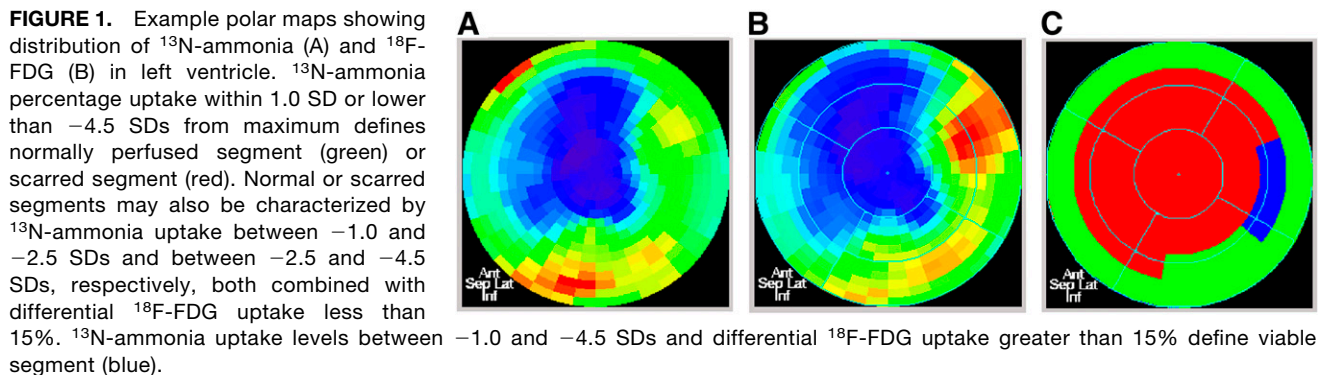
### PET

All patients underwent cardiac PET studies (ECAT HR+; Siemens/CTI) after a fasting period of 8 h. A glucose load (80 g) was given to patients before the acquisition of a 15-min transmission scan for the correction of photon attenuation. Cardiac dynamic PET images (twelve 10-s images, four 30-s images, two 300-s images, and one 600-s image) were obtained under resting conditions after a mean 20-s intravenous administration of  $^{13}\text{N}$ -ammonia (mean activity, 707 MBq; 9.25 MBq/kg) diluted in 10 mL of saline solution. After the plasma glucose level had decreased, patients were injected with an average activity of 555 MBq of  $^{18}\text{F}$ -FDG for the evaluation of cardiac metabolism. After a repeated transmission scan, electrocardiography-gated images (8 frames per R-R interval) were obtained 40 min after the injection of labeled glucose analog.

### Image Analysis

Based on the MunichHeart software for cardiac analysis (25), 2 polar maps of the left ventricle were generated for, respectively,  $^{13}\text{N}$ -ammonia and  $^{18}\text{F}$ -FDG. Ischemic (necrotic and viable) and normally perfused areas were characterized by an automatic comparison between the percentage of mean counts in each of the 460 sectors of the  $^{13}\text{N}$ -ammonia polar map (normalized to the maximum counting rate of 5% of the brightness sectors) and the corresponding sectors of the normalized  $^{18}\text{F}$ -FDG polar map. Both maps were divided into 9 segments, which were classified as normal, viable, or scarred. When a mix of ischemic and normal sectors was found in the same segment, the assigned category was based on the type of tissue with the greatest segmental extent. The number of necrotic, viable, and normally perfused segments was calculated on the polar map together with infarct size and viable area, respectively, representing the percentage extent of scarred and viable tissue (Fig. 1).

A quantitative analysis of MBF in the 9-segment polar map was then performed on the basis of a well-known 3-compartment model (18,19). The mean values of MBF in normal, viable, and scarred segments were expressed as mL/min/g of tissue and were compared with the MBF values of a reference database (Fig. 2). The contractile function of myocardium was assessed by analysis of LVEF and end-diastolic volume, both calculated on  $^{18}\text{F}$ -FDG gated images with the automated quantitative gated SPECT soft-



ware of Cedars-Sinai (26). A comparison of LVEF values calculated with gated PET and echocardiography was also attempted.

### Data Analysis

The mean number of scarred, viable, and normal segments and the mean infarct size and mean viable area were compared within each group of patients and among all patient groups, at baseline and 3, 6, and 12 mo after AMI. At the same intervals, an intra- and intergroup comparison of MBF and cardiac function parameters was also attempted. The 2 group B patients who had coronary occlusion and the group C patient with heart transplantation were excluded from the study after the clinical events. All comparative analyses of semiquantitative (number of ischemic segments, extent of damaged areas, and indices of cardiac motion) and quantitative (MBF) parameters among the 3 groups of patients were performed using the *t* test for unpaired data, whereas the intragroup analyses were accomplished with the *t* test for paired samples. A *P* value of less than 0.05 was considered significant.

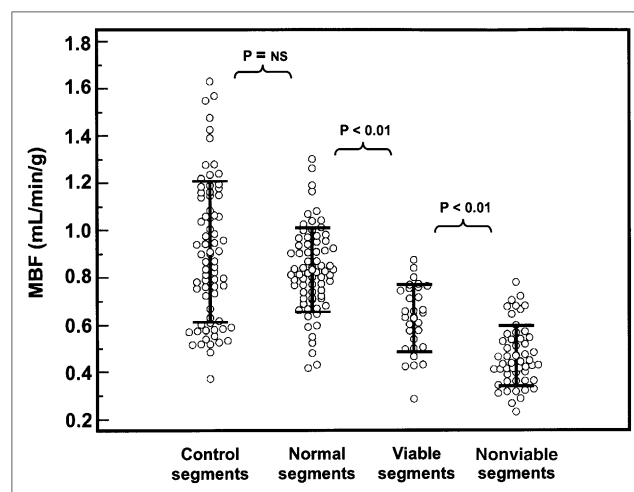
### RESULTS

No adverse events were reported during or immediately after cell administration. Over the 12-mo follow-up, there were no hospital admissions for angina or heart failure in group A, whereas 6 hospital admissions for angina were registered in group B (3 with a normal angiogram and 2 with an acute and chronic in-stent occlusion 4 and 9 mo, respectively, after AMI). Three admissions were also registered in group C (all patients had a normal angiogram and one underwent a transplantation for heart failure 6 mo after infarction).

The results of the semiquantitative analysis concerning the extent of myocardial damage at baseline and at different intervals during follow-up are reported in Table 1. At baseline, 63 of 135 segments (46.7%) were considered abnormal at automated analysis of the polar maps. No significant differences were observed in the number of pathologic segments (viable and scarred) or in the mean extent of infarct size and the viable area in the 3 groups before treatment. A constant decrease in the mean number of abnormal segments was observed during follow-up in group A ( $P < 0.05$ ), whereas a more irregular reduction was noted in groups B and C. Moreover, a significant difference was found between groups A and B at 6 and 12 mo ( $P < 0.01$ ). Infarct size significantly decreased only in group A ( $P < 0.05$  at 12 mo vs. baseline), despite a substantial

stability of the perfusion defect in group B and a patchy decrease in group C. The intergroup statistical analysis showed a significant difference in mean infarct size between groups A and B at 3, 6, and 12 mo and between groups B and C at 6 and 12 mo ( $P < 0.05$  for all comparisons). Besides a transient 3-mo increase ( $n = 12$ ) in the total number of viable segments in comparison to baseline ( $n = 9$ ), no differences in the number of viable segments or in mean viable area were found among patient groups.

Mean MBF significantly increased ( $P < 0.05$ ) in the infarct area of group A at 12 mo, whereas a progressive reduction occurring in group B ( $P = 0.01$  at 6 mo) was also associated with a decrease of MBF in nonischemic areas at 3 mo ( $P < 0.05$ ), as well as after 6 ( $P < 0.01$ ) and 12 mo of follow-up. Minimal changes in MBF were found in group C at the same intervals. At intergroup comparative analysis, the differences in mean MBF between groups A and B already were significant at 3 mo after AMI ( $P < 0.05$ ) and were steadily observed at 6 mo ( $P < 0.01$ ) and 12 mo ( $P < 0.05$ ). Moreover, a fainter but significant difference was seen between groups B and C at 6 mo ( $P < 0.05$ ) (Table 2).



**FIGURE 2.** Comparison among MBF values in normal, viable, and scarred segments of all patients enrolled in study and MBF values in control segments of reference database.

**TABLE 1**  
Semi-quantitative Analysis of Myocardial Damage

Patient no.	Baseline						3 mo			6 mo			12 mo		
	Abnormal segments*	Infarct size†	Viable area‡	Abnormal segments	Infarct size	Viable area	Abnormal segments	Infarct size	Viable area	Abnormal segments	Infarct size	Viable area	Abnormal segments	Infarct size	Viable area
<b>Group A</b>															
1	4 (1)	0.49	0.04	3 (0)	0.30	—	3 (0)	0.33	—	3 (0)	0.30	—	3 (0)	0.30	—
2	3 (1)	0.47	0.06	3 (0)	0.41	—	3 (0)	0.45	—	3 (0)	0.45	—	3 (0)	0.45	—
3	7 (0)	0.76	—	4 (1)	0.47	0.06	3 (0)	0.49	0.06	3 (0)	0.49	0.06	3 (0)	0.41	—
4	3 (0)	0.49	—	3 (0)	0.47	—	3 (0)	0.45	—	3 (0)	0.41	—	3 (0)	0.41	—
5	4 (0)	0.45	0.04	5 (2)	0.41	0.19	4 (1)	0.32	0.17	2 (0)	0.22	0.17	2 (0)	0.22	—
Mean ± SD	4.2 ± 1.6	0.53 ± 0.13	0.03 ± 0.03	3.6 ± 0.9	0.41 ± 0.07	0.05 ± 0.08	3.2 ± 0.4	0.41 ± 0.08	0.03 ± 0.08	2.8 ± 0.4	0.36 ± 0.10	0.03 ± 0.08	2.8 ± 0.4	0.36 ± 0.10	0.00 ± 0.00
<b>Group B</b>															
6†	4 (0)	0.53	0.04	5 (2)	0.57	0.16	—	—	—	—	—	—	—	—	—
7	5 (3)	0.35	0.18	3 (0)	0.49	—	4 (0)	0.49	—	4 (0)	0.45	—	4 (0)	0.45	0.04
8	5 (0)	0.57	—	5 (0)	0.57	—	6 (1)	0.57	0.04	5 (0)	0.45	0.04	5 (0)	0.45	—
9‡	4 (0)	0.57	—	6 (2)	0.41	0.16	4 (0)	0.53	—	—	—	—	—	—	—
10	5 (0)	0.65	—	3 (0)	0.61	—	6 (1)	0.57	0.04	4 (0)	0.64	0.04	4 (0)	0.64	—
Mean ± SD	4.6 ± 0.5	0.53 ± 0.11	0.04 ± 0.08	4.4 ± 1.3	0.53 ± 0.08	0.06 ± 0.09	5.0 ± 1.1	0.54 ± 0.04	0.02 ± 0.02	4.2 ± 0.4	0.51 ± 0.11	0.02 ± 0.02	4.2 ± 0.4	0.51 ± 0.11	0.00 ± 0.02
<b>Group C</b>															
11	2 (0)	0.30	—	6 (2)	0.41	0.12	3 (1)	0.26	0.08	3 (1)	0.22	0.08	3 (1)	0.22	0.08
12	5 (4)	0.12	0.49	3 (2)	0.16	0.37	5 (2)	0.24	0.25	2 (1)	0.18	0.25	2 (1)	0.18	0.15
13	4 (0)	0.41	—	4 (0)	0.37	—	3 (0)	0.30	—	3 (0)	0.33	—	3 (0)	0.33	—
14	3 (0)	0.41	—	4 (0)	0.49	—	4 (0)	0.46	—	4 (0)	0.41	—	4 (0)	0.41	—
15‡	5 (0)	0.76	—	6 (1)	0.76	0.04	6 (2)	0.57	0.16	—	—	0.16	—	—	—
Mean ± SD	3.8 ± 1.3	0.40 ± 0.23	0.10 ± 0.22	4.6 ± 1.3	0.46 ± 0.25	0.10 ± 0.15	4.2 ± 1.3	0.37 ± 0.14	0.09 ± 0.11	3.0 ± 0.8	0.28 ± 0.10	0.09 ± 0.11	3.0 ± 0.8	0.28 ± 0.10	0.05 ± 0.07

\*Number of scarred plus viable segments (viable segments in parentheses).

†Infarct size and viable area are expressed as percentage of total polar map area.

‡Patients excluded from study during follow-up due to reocclusion (patients 6 and 9) or cardiac transplantation (patient 15).

**TABLE 2**  
Quantitative Analysis of MBF in Infarcted and Noninfarcted Areas

Patient no.	MBF (mL/min/g) at...											
	Baseline		3 mo		6 mo		12 mo					
	Infarcted area	Noninfarcted area	Infarcted area	Noninfarcted area	Infarcted area	Noninfarcted area	Infarcted area	Noninfarcted area	Infarcted area	Noninfarcted area	Infarcted area	Noninfarcted area
<b>Group A</b>												
1	0.514	0.779	0.470	0.833	0.538	0.822	0.623	0.855				
2	0.531	0.921	0.544	0.909	0.513	0.783	0.583	0.801				
3	0.502	0.904	0.544	0.791	0.630	0.930	0.824	1.256				
4	0.399	0.856	0.410	0.934	0.425	1.016	0.573	1.019				
5	0.399	0.517	0.492	0.668	0.452	0.563	0.460	0.496				
Mean ± SD	0.47 ± 0.06	0.80 ± 0.17	0.49 ± 0.06	0.83 ± 0.11	0.51 ± 0.08	0.82 ± 0.17	0.61 ± 0.13	0.89 ± 0.28				
<b>Group B</b>												
6*	0.374	0.885	0.398	0.827	—	—	—	—				
7	0.432	0.806	0.397	0.730	0.351	0.591	0.336	0.685				
8	0.432	0.781	0.400	0.680	0.340	0.629	0.311	0.626				
9*	0.556	0.881	0.503	0.756	0.396	0.642	—	—				
10	0.416	0.814	0.335	0.522	0.334	0.534	0.399	0.681				
Mean ± SD	0.44 ± 0.07	0.83 ± 0.05	0.41 ± 0.06	0.70 ± 0.11	0.36 ± 0.03	0.60 ± 0.05	0.35 ± 0.05	0.66 ± 0.03				
<b>Group C</b>												
11	0.552	0.805	0.418	0.761	0.539	0.875	0.514	0.731				
12	0.577	0.845	0.587	0.870	0.566	0.677	0.770	0.974				
13	0.619	0.925	0.602	0.860	0.557	0.878	0.488	0.766				
14	0.490	1.022	0.407	0.812	0.453	0.800	0.448	0.738				
15*	0.424	0.629	0.428	0.848	0.440	0.667	—	—				
Mean ± SD	0.53 ± 0.08	0.85 ± 0.15	0.49 ± 0.10	0.83 ± 0.04	0.51 ± 0.06	0.78 ± 0.10	0.56 ± 0.15	0.80 ± 0.12				

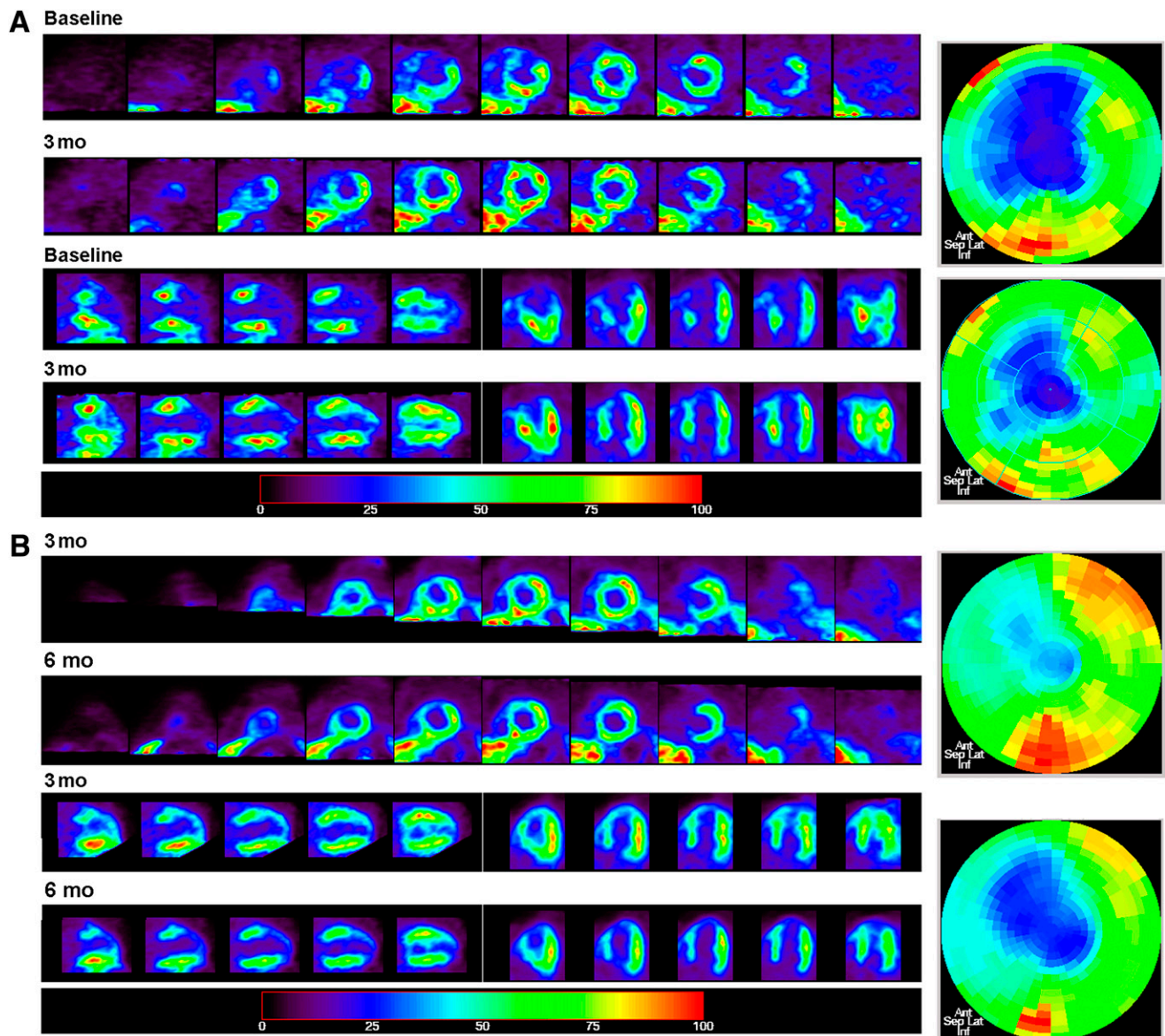
\*Patients excluded from study during follow-up because of reocclusion (patients 6 and 9) or cardiac transplantation (patient 15).

Concerning the analysis of gated PET-derived cardiac function parameters (Supplemental Table 2), no significant differences in mean baseline LVEF were found among groups, although slightly higher values were observed in groups A and C. During follow-up, no significant changes in mean LVEF were found in group A, whereas a nonnegligible decrease (tending to statistical significance at 12 mo;  $P = 0.07$ ) was observed in group B. The difference in mean LVEF between groups A and B was proven to be significant already at 3 mo ( $P = 0.02$ ). The mild reduction of LVEF observed in group C was not statistically significant at intra-group or intergroup comparative analysis. In accord with these findings, a significant increase of mean end-diastolic volume was observed only in group B ( $P = 0.03$  at 3 mo); a

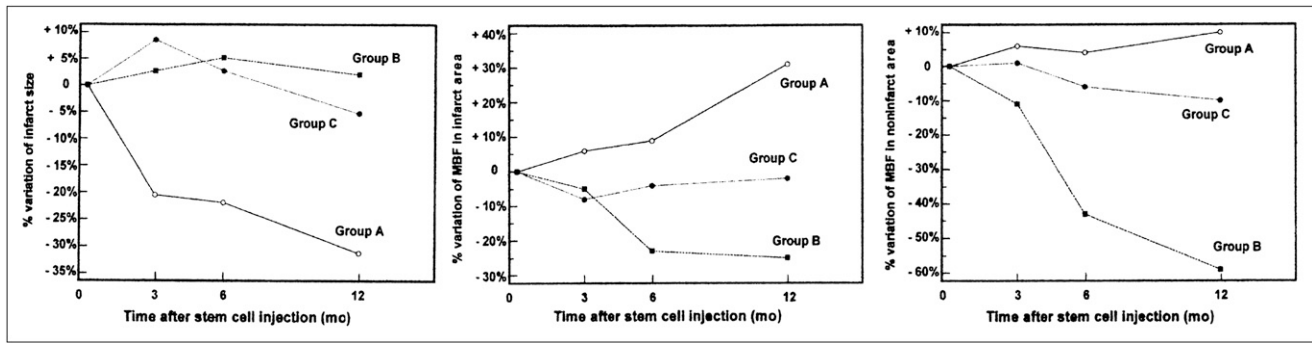
significant difference in mean end-diastolic volume was also noted between groups A and B at 6 mo ( $P = 0.05$ ) and 12 mo ( $P < 0.01$ ). Despite its statistical significance ( $P < 0.01$ ), a weak correlation between LVEF values obtained by echocardiography and gated PET was found ( $R = 0.57$ ). In this connection, the good correlation obtained at baseline ( $R = 0.77$ ) was counterbalanced by the lack of a significant relationship 12 mo after AMI ( $R = 0.48$ ).

## DISCUSSION

The last decade has seen the start of many clinical trials to assess the effect of intracoronary injection of stem cells on cardiac repair after AMI (2–17). Nevertheless, the results obtained are still equivocal and suggest that differ-



**FIGURE 3.**  $^{13}\text{N}$ -ammonia serial images and polar maps of 2 patients of groups A and B. (A) Shrinkage of perfusion defect in patient 3 at 3 mo after injection of bone marrow-derived cells. (B) Enlargement of perfusion defect in patient 9, who had in-stent chronic occlusion between 3 and 6 mo after therapy with peripheral blood-derived cells. Relative decrease in ammonia uptake is consistent with reduction of MBF also in non-infarct-related areas.

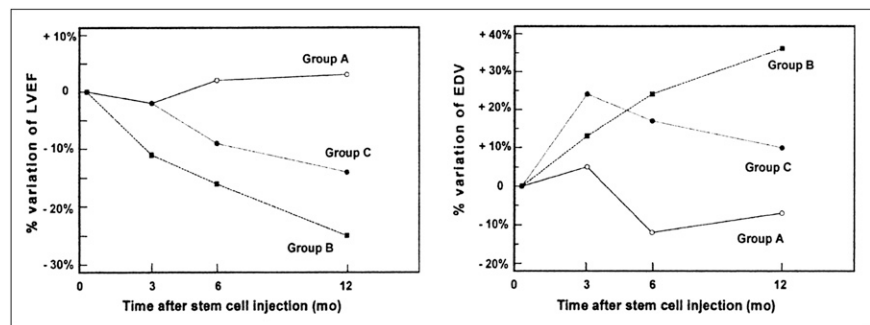


**FIGURE 4.** Mean percentage variation in infarct size and MBF in patients of the 3 groups during follow-up.

ences in the type and number of cells injected, administration mode, and follow-up time may hamper a proper assessment of the efficacy of this novel treatment (17,27,28). Different imaging techniques have been used to assess the changes in cardiac function (3,4,6–8,11,13–15,17) and myocardial perfusion after stem cell transplantation (5,8–11). However, besides the evaluation of cardiac function obtained in all studies by means of LVEF estimation along with the measurement of other kinetic parameters, changes in myocardial flow have been assessed mostly with an automated quantification of infarct size calculated from MRI (7,8,14), perfusion scintigraphy (2,5,6,10,17), and  $^{18}\text{F}$ -FDG PET images (29) or with a visual analysis of  $^{99\text{m}}\text{Tc}$ -sestamibi uptake in necrotic segments (16,30,31). The quantitative analysis of MBF already attempted with  $^{11}\text{C}$ -acetate (8) but performed for the first time (to our knowledge) with ammonia in our study could provide a more accurate assessment of changes in cardiac perfusion in patients treated with bone marrow- or plasma-derived endothelial progenitors, as well as in patients who receive standard medical therapy for AMI. Additional information about metabolism (3,6,32–34) and function in repaired myocardial tissue was obtained with the contemporary evaluation of cardiac  $^{18}\text{F}$ -FDG uptake and kinetic parameters derived from electrocardiography-gated  $^{18}\text{F}$ -FDG images.

Similar to most previous studies concerning changes in myocardial perfusion in patients treated with intracoronary injection of bone marrow-derived stem cells and with standard therapy after infarction (Supplemental Table 3), a mean reduction of infarct size was observed for both

group A and group C also in our study (Table 1; Fig. 3A). However, a significant shrinkage of the defect was observed only for group A at 12 mo and seemed to be related to the more constant reduction of necrosis in these patients (Fig. 4). The more variable changes in the perfusion defect size observed in group C rather suggest that shrinkage of the necrotic area is less predictable in patients treated with conventional therapy. These results are supported by the analysis of MBF calculated in infarcted territories (Table 2). A significant increase in MBF mean values (even approximating MBF values of our reference database) was observed in the scarred regions of group A but not in group C patients, whose regional perfusion remained globally unchanged (Fig. 4). The results of the analysis of cardiac perfusion in group B raise an interesting issue. Indeed, we were not able to confirm the significant reduction of infarct size reported by other authors who used peripheral blood-derived stem cells (14,31), whereas a further decrease of MBF in necrotic areas, along with only mild shrinkage of the perfusion defect, was found in these patients. Various hypotheses (35–37) may explain the lack of efficacy of peripheral blood-derived CD133+ cells. These include undetectable levels of the CD133+/CD34- endothelial progenitor with a greater clonogenic potential, a granulocyte colony-stimulating factor-mediated reduction in homing capacity of prevalent CD133+CD34+ immunophenotype, and a 100-fold reduction in the number of peripheral blood-derived cells injected in comparison with the above studies. However, the significant decrease of



**FIGURE 5.** Mean percentage variation in LVEF and end-diastolic volume in patients of the 3 groups during follow-up.

MBF also observed in non-infarct-related cardiac areas, and the restenosis that occurred in 2 patients (Fig. 3B), rather resemble the adverse effects on the coronary vessels already described by Kang et al. (5) and related to granulocyte colony-stimulating factor-induced neointimal hyperplasia.

The search for viable tissue was performed to assess possible differences in cardiac metabolism induced by neoangiogenesis stimulated by different therapies. Although a relative increase in viable tissue ( $\leq 20\%$  of all pathologic segments) was found 3 mo after AMI, no differences in the mean number of viable segments or in the extent of the viable area were observed among the groups. Moreover, the finding of perinecrotic viable tissue in patients who later experienced an in-stent occlusion was not consistent with the relationship between the lack of viability in the infarct area and the development of a restenosis suggested by Assmus et al. (33). According to the usual time course of myocardial viability after AMI (38), a progressive reduction in the number of viable segments was observed at 6 and 12 mo, without any significant differences among the groups differently treated (Table 1).

Other interesting issues are the simultaneous assessment of cardiac function provided by gated  $^{18}\text{F}$ -FDG PET images (Supplemental Table 2) and the arguable relationships among cardiac kinetic parameters, the extent of ischemic areas, and global MBF values. The increase of baseline LVEF ( $\leq 3\%$ ) and the corresponding reduction of end-diastolic volume ( $\leq 7\%$ ) observed at 12 mo in patients treated with bone marrow-derived stem cells are consistent with the shrinkage of damaged area and with the improved MBF (Figs. 4 and 5). Similarly, the lack of improvement in kinetic parameters in group C may be related to an unchanged infarct size and to stable MBF values found in the infarct zone. Otherwise, the further and marked impairment of cardiac function observed in group B may not be fully explained by the lack of significant shrinkage of the perfusion defect (seen also in group C) or by the reduction of MBF found in the necrotic area. The decrease of MBF observed even in unrelated-infarct cardiac territories could be responsible for the major decrease of cardiac function found in these patients (Table 2; Fig. 4). The weak, although significant, correlation observed between LVEF estimated with  $^{18}\text{F}$ -FDG PET and echocardiography may have been caused by the well-known differences between these techniques in imaging of a remodeled left ventricle (39). The lack of contrast-enhanced or 3-dimensional echocardiography might be considered a methodologic pitfall because the use of these techniques could allow a more reliable comparison with gated PET data.

The major drawback of the study remains the small number of patients enrolled in the trial, because it reduces the clinical impact of our results and does not provide straightforward information about the effects of different types of endothelial progenitors on cardiac repair. Nevertheless, our primary endpoint was to evaluate the feasibility of simultaneous assessment of cardiac flow and metabolism

in a novel field of cardiology research. In view of the general agreement of our data with the results of previous studies and with the clinical outcome of our patients, the results of this first experience with combined  $^{13}\text{N}$ -ammonia and  $^{18}\text{F}$ -FDG cardiac PET have fully satisfied our goal.

## CONCLUSION

Our results indicate that a combined  $^{13}\text{N}$ -ammonia/ $^{18}\text{F}$ -FDG PET study may provide a noninvasive assessment of the physiopathologic changes in cardiac perfusion and metabolism induced by stem cell therapy after AMI.

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