

## Chapter 2

# **PET for Evaluation of Differential Myocardial Perfusion Dynamics after VEGF Gene Therapy and Laser Therapy in End-Stage Coronary Artery Disease**

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## Abstract

### Aim

The purpose of this study was to appraise the value of PET in the assessment of the effect of supposedly proangiogenic new therapies such as gene therapy with vascular endothelial growth factor (VEGF) gene and endomyocardial laser therapy.

### Methods

Thirty-five patients with end-stage coronary artery disease and class III (Canadian Cardiovascular Society) angina were included. Myocardial ischemia was evaluated with dipyridamole PET scanning and exercise tolerance with bicycle ergometry. Ten patients were treated with naked plasmid DNA encoding for human VEGF165 (VEGF) and 12 patients were treated with laser therapy (direct myocardial revascularization [DMR]) using an electromechanical mapping system. Thirteen patients were treated with standard medical therapy (control).

### Results

In both active treatment groups, angina was reduced in most subjects, except in 2 VEGF and 5 DMR patients. In the control group, no improvement in anginal classification was found, except in 3 subjects. On the PET scan, solely in the VEGF group, the stress perfusion was significantly improved (from  $57 \pm 33$  to  $81 \pm 55$  mL/min/100 g;  $P=0.031$ ). Furthermore, in the VEGF group, the number of ischemic segments was reduced from  $274 \pm 41$  to  $234 \pm 48$  segments ( $P=0.004$ ) but not in the DMR group (from  $209 \pm 43$  to  $215 \pm 52$  segments) or in the control group (from  $218 \pm 18$  to  $213 \pm 28$  segments). Bicycle exercise duration showed slight nonsignificant changes in the VEGF group (from  $3.6 \pm 2.0$  to  $4.6 \pm 2.1$  min), in the DMR group (from  $5.1 \pm 1.5$  to  $4.7 \pm 1.3$  min), and in the control group (from  $3.3 \pm 1.8$  to  $3.5 \pm 2.3$  min).

### Conclusion

PET showed that intramyocardial gene therapy with the human VEGF165 gene in contrast to laser DMR treatment effectively reduces myocardial ischemia.

## Introduction

Coronary artery disease (CAD) is a progressive disease with a high morbidity and mortality. Conventional treatment consists of medical therapy as the first-line strategy, followed by percutaneous or surgical revascularization. An increasing number of patients with refractory angina cannot be treated with these conventional interventions<sup>1,2</sup>. In this group, alternative treatment modalities such as external balloon counter-pulsation, chronic intermittent urokinase therapy, and electrical neurostimulation have been explored<sup>3</sup>. In addition, gene therapy with angiogenic growth factors and endomyocardial laser treatment have been developed as potential new treatment modalities<sup>4</sup>.

Initial reports on angiogenic gene therapy required a thoracotomy and general anesthesia for intramyocardial gene administration<sup>5-7</sup>. Recent studies report a percutaneous application alternative<sup>8,9</sup>. These uncontrolled gene therapy trials have shown promising results through a reduction in anginal complaints and in myocardial ischemia and an improvement in exercise duration<sup>5-8</sup>.

Imaging of the supposedly newly formed collaterals remains difficult<sup>10</sup>. Surrogate measurements such as the Rentrop score and relative perfusion imaging with SPECT have yielded promising results<sup>5,11</sup>. To our knowledge, no data on myocardial perfusion with PET in patients treated with vascular endothelial growth factor (VEGF) gene therapy have been published so far. Since PET has the advantage of absolute quantification of myocardial perfusion and is not disturbed by attenuation artifacts like SPECT, we hypothesize that PET perfusion imaging can be a powerful tool in evaluation of new therapies in patients with refractory angina pectoris. Patients receiving either VEGF gene therapy or laser therapy (direct myocardial revascularization [DMR]) were compared with patients who underwent standard medical treatment.

## Materials and methods

### Patients

Subjects (age, >18 y) with stable exertional angina who were not candidates for surgical or percutaneous revascularization were eligible. In addition, they should have documented areas of underperfused but viable myocardium on a previous rest–dipyridamole stress PET scan. The presence of at least 15% of the myocardium with a low perfusion reserve (<140%) was a requirement for inclusion. Subjects were excluded if coronary artery bypass graft surgery was performed within 90 d before study inclusion. Additional exclusion criteria were pregnancy, lactation, or the use of inadequate contraception (premenopausal women); evidence or history of cancer, fundoscopic evidence of diabetic retinopathy, or age-related macular degeneration; other severe concurrent illness (e.g., active infection, severe congestive heart failure), left ventricular ejection fraction <20% (obtained by echocardiogram or radioisotope multiple-gated acquisition scan), significant aortic stenosis, inability to undergo

cardiac catheterization or nuclear testing, and an inability to follow the protocol or comply with follow-up requirements. Then they were randomly assigned to standard medical therapy (control) or DMR or to open-label VEGF gene therapy.

### **Catheterization procedures**

Patients were prepared and draped for a standard coronary catheterization procedure. A standard coronary and left ventricular angiogram was performed. Thereafter, an electro-mechanical mapping catheter was introduced. The NOGA system is a multielectrode catheter system (Biosense–Webster), which was developed for simultaneous measurement of local electrical (voltage) and mechanical (linear local shortening) activity in the heart (Cordis). This enables the creation of 3-dimensional voltage as well as linear local shortening (as an indicator of wall motion) maps of the left ventricle. In this way, areas of infarction (decreased mechanical and electrical activity), ischemia (decreased mechanical and normal electrical activity), and normal myocardium can be distinguished. All NOGA maps were divided in 9 segments for evaluation of mean voltage and linear local shortening using the company software program. After mapping of the left ventricle, either a NOGA-DMR (holmium–yttrium aluminum garnet laser) or NOGA-injection catheter was introduced. All maps consisted of at least 50 points after filtering. The Biosense Injection Catheter contains a retractable needle that can be controlled from the injection handle. It was designed to inject fluid into the myocardium. The tip is equipped with a Biosense location sensor for navigation and mapping using the Biosense system. This navigation system facilitates manipulation of the catheter to a predefined myocardial area.

Patients in the VEGF gene therapy group were treated with naked DNA encoding for VEGF165 (Kalid Islam; Gene Therapy Center). A total dose of 500  $\mu$ g divided over 10 endomyocardial injections with a volume of 0.3 mL each was administered with the deflectable injection catheter (NOGA-InjectionStar; Biosense–Webster). The injection needle was inserted into the myocardium after stabilization of the catheter tip (loop stability <2). The plasmid solution was injected only after premature ventricular contractions were seen following needle insertion.

Patients treated with laser DMR received 20–30 laser holes (2 J per pulse) using the NOGA direct myocardial revascularization system (Biosense–Webster). All other patients, including the control group, received optimal medical treatment. Follow-up visits were on a weekly basis during the first month and after 3 mo. The 3-mo visit was also the final follow-up visit. Patients in the control group received a NOGA-DMR treatment after completion of the follow-up. In all patients, DMR laser holes as well as VEGF gene injections were aimed at ischemic as well as the immediately adjacent nonischemic border zone.

### **PET studies**

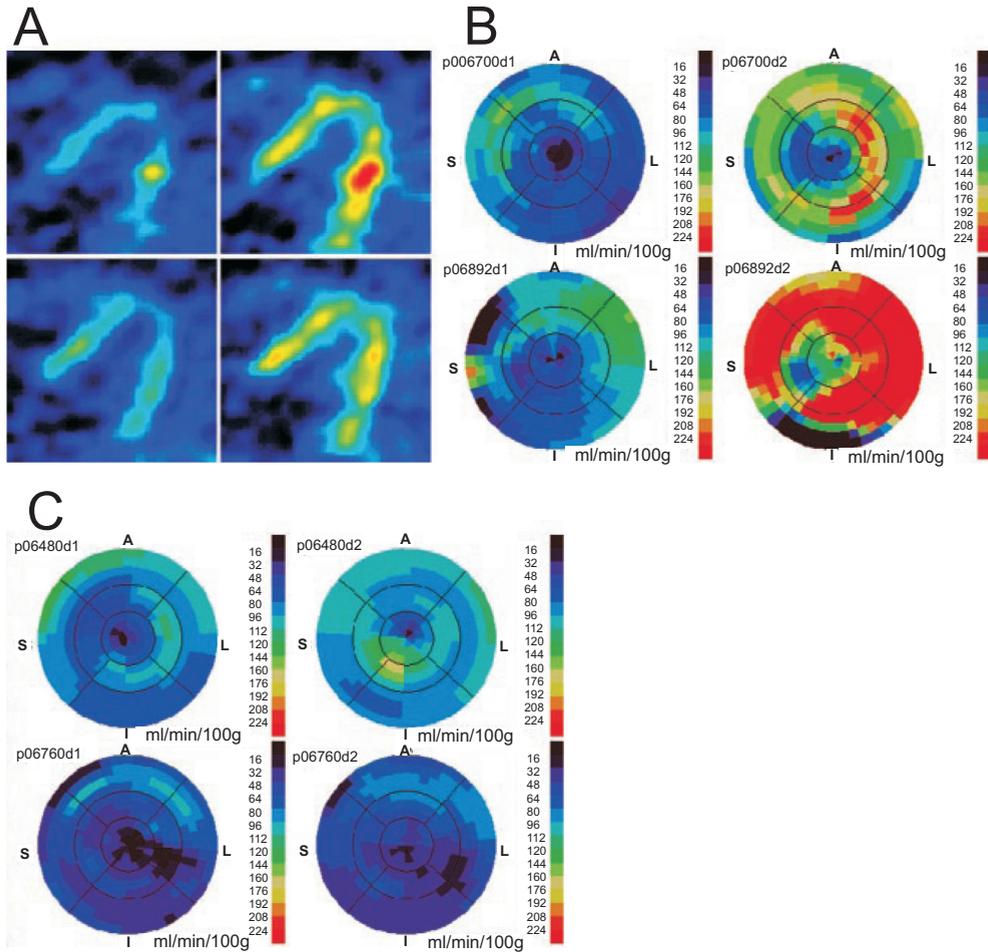
All patients underwent a PET scan before inclusion and at the end of the 3-mo follow-up period. The PET studies were performed after patients had refrained from antianginal medical therapy for 5 plasma half-lives (up to several days depending on the drug regimen) and

caffeinated beverages for 24 h before the studies. Patients received a carbohydrate-rich meal before the PET scan. Patients were positioned in a 951 Siemens (ECAT) positron camera (Siemens AG), which images 31 planes simultaneously over 10.8 cm (axial field of view). Subjects were positioned with the help of a rectilinear scan. Data were automatically corrected for accidental coincidence and dead time. Photon attenuation was measured using a rectilinear external ring source filled with  $^{68}\text{Ge}/^{68}\text{Ga}$ . With the attenuation correction used for this study (the 951 2-dimensional system), the problem of scatter is estimated to be in the range of a few percent. Patients were constantly monitored with 12-lead electrocardiography, and blood pressure was measured automatically every 10 min and every minute during dipyridamole infusion. Myocardial blood flow was studied as previously described using  $^{13}\text{N}$ -ammonia as the tracer<sup>12</sup>. Dynamic rest imaging was started at the time of  $^{13}\text{N}$ -ammonia injection (370 MBq) and continued for 15 min (frames 12 x 10 s, 1 x 2 min, 1 x 4 min, 1 x 7 min). Dipyridamole stress imaging was performed by infusing dipyridamole (0.56 mg/kg body weight in 4 min). Imaging was done by injecting 370 MBq  $^{13}\text{N}$ -ammonia 6 min after the start of dipyridamole infusion and continued for 15 min (frames 12 x 10 s, 1 x 2 min, 1 x 4 min, 1 x 7 min). After this, myocardial glucose uptake was studied with  $^{18}\text{F}$ -FDG during hyperinsulinemic-euglycemic clamping. The  $^{18}\text{F}$ -FDG imaging was done 5 min after injecting 185 MBq  $^{18}\text{F}$ -FDG and continued for 35 min (frames 8 x 15 s, 4 x 30 s, 1 x 1 min, 1 x 5 min, 1 x 10 min, 1 x 15 min). The duration between each of the PET studies was at least 20 min, with correction for remaining activity. The Hutchins method was used for  $\text{NH}_3$  quantification<sup>13</sup>. The Patlak method was used for FDG quantification<sup>14</sup>.

The data from the dipyridamole  $^{13}\text{N}$ -ammonia and  $^{18}\text{F}$ -FDG studies were corrected for remaining activity by subtracting the last frame of the preceding study and corrected for the isotope half-life of  $^{13}\text{N}$ -ammonia. After this subtraction procedure, data for each study were reoriented to 10 short-axis images using a manually drawn long axis in the left ventricle. The myocardium in the 10 slices was divided into 48 segments ( $7.5^\circ$  each). Using the maximal activity, time-activity curves were established in all segments of all slices. The blood pool was defined in 3 slices near the base, the average of which was used to calculate a single blood-pool time-activity curve. For each of the 480 segments, myocardial blood flow was calculated using a curve fit over 120 s as previously described<sup>12</sup>.

For each individual segment, rest and dipyridamole-stress myocardial perfusion, and FDG metabolism were calculated. From these data a parametric polar map for each modality was constructed. An example of PET imaging and polar map construction is shown in figures 1A and 1B.

A flow increase during dipyridamole stress of  $<40\%$  from baseline was considered to be abnormal. After excluding infarcted areas ( $^{18}\text{F}$ -FDG uptake  $<25\%$  of the maximal uptake), flow and flow reserve were analyzed for each of the remaining segments using a customized software program as previously described<sup>15</sup>. The number of ischemic segments, global or overall perfusion, and regional ischemic perfusion were analyzed. During analysis the observers were unaware of treatment assignment and sequence.



**Figure 1.** (A) Static image of long-axis views of NH3 PET scan at rest (left) and during dipryidamole stress (right). Top 2 panels, at baseline; bottom 2 panels, at follow-up. (B) Polar map reconstruction of scans shown in A: VEGF-treated patient showing marked improvement in perfusion and reduction of anginal complaints. Top 2 panels, at baseline; bottom 2 panels, at follow-up; left-hand panels, rest; right-hand panels, dipryidamole stress. (C) Polar map of DMR patient showing no improvement in perfusion images or in anginal complaints. Top 2 panels, at baseline; bottom 2 panels, at follow-up; left-hand panels, rest; right-hand panels, dipryidamole stress.

### Additional efficacy measurements

Anginal complaints were scored according to the Canadian Cardiovascular Society (CCS) classification system. During a standard interview an independent interviewer assessed the CCS classification. Standard supine bicycle ergometry was performed at baseline and at the end of the follow-up period. The workload was increased with 10 W/min, until ischemia was present or until patients stopped because of fatigue, dyspnea, angina, or other complaints.

In addition to the CCS classification, quality of life was assessed with the RAND-36 Item Health Survey (also known as the MOS SF-36). The RAND-36 measures 8 health concepts: physical functioning, bodily pain, role limitations due to personal or emotional problems, emotional well being, social functioning, energy or fatigue, and general health perceptions. The RAND-36 has been validated and applied in many countries. Recently, the RAND-36 has been translated and validated in The Netherlands<sup>16,17</sup>.

In addition, quality-of-life assessment was completed with disease-specific measurements: the Multidimensional Fatigue Index (MFI-20), which contains 5 subscales (general, physical, activity, motivation, cognition) and the feelings-of-disability subscale of the Medical Psychological Questionnaire for Heart Patients (MPVH).

The MFI-20 consists of 20 items and 5 subscales, each with a range from 4 to 20 and a total score range from 20 to 100. A high score indicates high fatigue<sup>18</sup>. The feelings-of-disability subscale of the MPVH consists of 11 items, with a range from 11 to 33. High scores indicate greater feelings of disability.

### Statistics

Continuous data are presented as mean  $\pm$  SD. Categorical data are presented as percentage and count of each category. Treatment effects were analyzed comparing the absolute changes in end-point variables. In the primary analysis, for the continuous variables, treatment groups were compared using 1-way ANOVA if normally distributed or using the Kruskal–Wallis test if the distribution was skewed. For categorical data, treatment comparisons were made using the Fisher exact test or  $\chi^2$  tests. The normality of data was assessed by using the Shapiro–Wilk test for normality.

In addition, within a treatment group, changes were assessed using a paired *t* test or a Wilcoxon signed rank test. Baseline comparability was assessed using the same statistical tests as for the treatment effects.

A *P* value of  $<0.05$  was considered statistically significant. All analyses were performed using commercially available computer software (Statistical Analysis System version 6.12; SAS Institute).

To evaluate the magnitude of quality-of-life assessment change over time within each group, effect sizes were calculated on the basis of paired observations. Mathematically, the effect size is the difference (change) in a score within subjects divided by the pooled SD for that score. The interpretation of these effect sizes was defined as proposed by Cohen<sup>19,20</sup>: an effect size  $\leq 0.20$ , no effect on dimension of interest; an effect size between 0.20 and 0.50, a small effect; an effect size between 0.50 and 0.80, a medium effect; and an effect size  $\geq 0.80$ , a large effect.

### Ethical considerations

Initially, a placebo-controlled gene therapy protocol was proposed to the national Central Committee on Research Involving Human Subjects. At that stage, no permission was granted for a placebo-controlled study, but permission was granted solely for an open-label treatment

with the VEGF plasmid. Concomitantly, the institutional review board gave permission for the laser study with DMR or the control. All patients were recruited from the same source and sequentially assigned to the VEGF gene therapy group or the laser study groups. All patients referred to our center for experimental treatment of their end-stage CAD underwent a PET scan. Based on the results of the PET scan, patient eligibility was assessed; in case of eligibility, patients were randomly assigned to open-label VEGF gene therapy or to laser DMR or care as usual.

## Results

Sixty patients were initially referred to our center for refractory angina pectoris (CCS class III angina without interventional options) and evaluated by PET. From this group, 35 patients were included on the basis of the results of the inclusion PET scan. Patients were treated either with standard therapy (control;  $n = 13$ ), percutaneous myocardial laser revascularization (DMR;  $n = 12$ ), or percutaneous angiogenic gene therapy with VEGF165 plasmid ( $n = 10$ ).

No differences in age or sex were present (table 1). All patients were on optimal medical treatment. No differences with regard to risk factors or the extent of CAD were present (table 1).

Myocardial perfusion was evaluated by PET at rest and during dipyridamole stress. In all groups, neither rest- nor stress global perfusion changed during follow-up (table 2). However, in the VEGF group but not in the DMR or control group, the perfusion during dipyridamole significantly improved in the ischemic area (from  $57 \pm 33$  to  $81 \pm 55$  mL/min/100 g;  $P = 0.031$ ; table 2). Furthermore, in the VEGF group, the number of ischemic segments on the PET scan was reduced from  $274 \pm 41$  to  $234 \pm 48$  ( $P = 0.004$ ). The number of ischemic segments was not different after DMR (from  $209 \pm 43$  to  $215 \pm 52$  segments) or in the control group (from  $218 \pm 18$  to  $213 \pm 28$  segments).

In the VEGF group, of the initially ischemic segments,  $53 \pm 9$  improved, whereas  $13 \pm 3$  new ischemic segments were identified at follow-up. In the DMR group, these values were  $59 \pm 24$  and  $69 \pm 21$ , and, in the control group, these values were  $130 \pm 24$  and  $127 \pm 21$ , respectively.

Myocardial viability was evaluated by 18F-FDG PET scans. Maximal, standardized 18F-FDG uptake in all patients was  $68 \pm 4$   $\mu\text{mol/L/min/100 g}$  at baseline and  $70 \pm 5$   $\mu\text{mol/L/min/100 g}$  at follow-up. At follow-up, a new (previously nonexistent) infarct was found in 1 control patient and in 1 DMR patient. The infarct areas as diagnosed at baseline did not significantly change from  $48 \pm 11$  to  $37 \pm 13$  segments for the group as a whole or for the individual groups (data not shown). The 18F-FDG metabolism between the ischemic ( $50 \pm 3$   $\mu\text{mol/L/min/100 g}$ ) and nonischemic ( $56 \pm 4$   $\mu\text{mol/L/min/100 g}$ ) areas was not different at baseline or at follow-up (ischemic,  $55 \pm 3$   $\mu\text{mol/L/min/100 g}$ ; nonischemic,  $55 \pm 3$   $\mu\text{mol/L/min/100 g}$ ).

**Table 1.** Baseline characteristics

Characteristic	Control	DMR	VEGF
	(n = 13)	(n = 12)	(n = 10)
Age* (y)	64 ± 5	60 ± 8	63 ± 8
Sex (M/F)	9/4	11/1	9/1
Hypercholesterolemia	12	11	10
Hypertension	3	7	4
Diabetes	0	3	4
Smoking	2	2	1
Previous MI*	1.3 ± 0.54	1.8 ± 0.4	1.1 ± 0.5
Previous PCI*	1.2 ± 1.9	1.8 ± 2.0	1.1 ± 1.6
Previous CABG*	0.8 ± 0.6	1.1 ± 1.1	1.0 ± 0.8
1-Vessel disease	2	2	0
2-Vessel disease	6	6	3
3-Vessel disease	5	4	7
Long-acting nitrates	9	9	8
Calcium antagonists	11	9	9
β-Blockers	12	11	8
ACE Inhibitors	6	3	3
Statins	11	12	10
Diuretics	3	4	5
Aspirin	7	9	5
Coumarins	6	3	4

\*Values are mean ± SD.

MI: myocardial infarction; PCI: percutaneous coronary intervention;

CABG: coronary artery bypass graft; ACE: angiotensinconverting enzyme.

**Table 2.** PET myocardial perfusion data

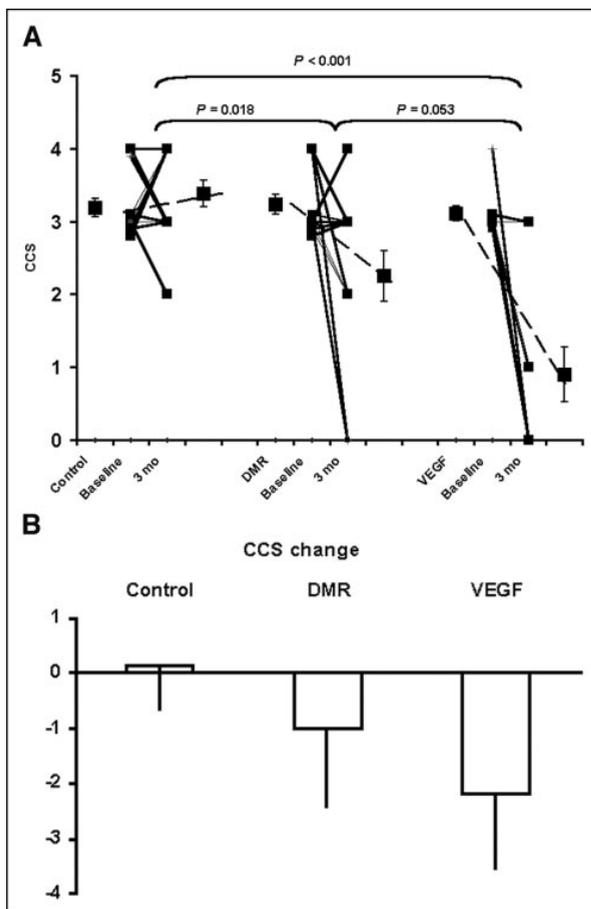
Global perfusion (mL/min/100 g tissue)			Regional perfusion in ischemic area (mL/min/100 g tissue)		
Group	Baseline	Follow-up	Group	Baseline	Follow-up
Control			Control		
Rest	96 ± 20	89 ± 29	Rest	78 ± 23	79 ± 30
Stress	122 ± 24	129 ± 57	Stress	93 ± 23	111 ± 54
DMR			DMR		
Rest	90 ± 20	99 ± 46	Rest	81 ± 16	83 ± 36
Stress	115 ± 27	117 ± 51	Stress	92 ± 23	106 ± 54
VEGF			VEGF		
Rest	84 ± 23	79 ± 28	Rest	63 ± 23	57 ± 37
Stress	111 ± 47	112 ± 59	Stress	63 ± 21	81 ± 55*

\*P = 0.05.

Values are mean ± SD.

In the control group, 5 patients demonstrated no change in anginal complaints. Three of 13 patients experienced a reduction and 5 patients experienced an increase in complaints after 3 mo of optimal drug treatment. In the DMR group, 7 of 12 patients experienced a reduction and 1 experienced an increase in anginal complaints. In the VEGF group, 8 of 10 patients experienced an improvement up to a level that 5 patients did not have any limitations in ordinary daily activities. The other 2 patients had no improvement or worsening of their anginal complaints (figure 2).

Exercise tolerance was evaluated with standard bicycle ergometry. Exercise duration showed slight nonsignificant changes in the VEGF group (from  $3.6 \pm 2.0$  to  $4.6 \pm 2.1$  min), the DMR group ( $5.1 \pm 1.5$  vs.  $4.7 \pm 1.3$  min), and the control group ( $3.3 \pm 1.8$  vs.  $3.5 \pm 2.3$  min).



**Figure 2.** (A) CCS angina pectoris classification at baseline and after 3 mo of follow-up in 3 groups (control, standard therapy; DMR, laser treatment; VEGF, VEGF gene therapy). Changes were significantly different between the 3 groups ( $P < 0.001$ ). (B) Change in CCS class in 3 individual groups. Change in VEGF gene therapy group was significantly different from that of other 2 groups ( $P < 0.05$ ).

Quality-of-life assessment was performed using the RAND-36 questionnaire. The mean baseline and posttest scores are represented in table 3 together with effect sizes. At the end of the study, beneficial changes in both the DMR- and the VEGF-treated patients, but not in the control group, were found in health-related quality-of-life dimensions.

**Table 3.** Quality-of-life aAssessments

Dimension	Control		DMR		VEGF	
	Baseline	3 mo	Baseline	3 mo	Baseline	3 mo
RAND-36						
Physical functioning	23	34*	36	58*	23	44*
Social functioning	40	40	57	65	25	52*
Role limitation physical	0	0	8	35*	4	25
Role limitation emotional	20	0	50	78	29	43
Mental health	51	41	59	66	49	60*
Vitality	34	41	38	55*	24	46*
Bodily pain	29	33	40	61*	26	57*
General health perception	27	31	31	47*	26	36
Perceived change in health	20	20	33	69	13	63*
MPVH, feelings of disability	34	35	34	31*	35	30*
MFI, fatigue overall score	77	75	71	58*	79	70*

\*Large changes in dimension according to effect sizes proposed by Cohen<sup>19,20</sup> ( $\geq 0.80$ ) are indicated.

Using the effect sizes as previously described, the overall effect in the VEGF group was large<sup>19,20</sup>. In the VEGF group, all changes in each of the dimensions were in the same direction as in the DMR group. However, the overall effect was more toward a medium level. Quality-of-life assessment according to the RAND-36 questionnaire revealed large effects on physical functioning (all 3 groups), social functioning (VEGF group only), mental health (VEGF group only), vitality (VEGF group only), pain perception (DMR and VEGF groups), and general health (DMR group only) and received a change in health (DMR and VEGF groups). Within each of the 2 treatment groups, the observed changes were highly significant. Between the 2 treatment groups, however, no statistical differences in relative changes were found, except for the feelings of disability score (MPVH) (table 3). No significant relationship was found between the improvement in anginal complaints and the improvement in myocardial perfusion. Patients without improvement in anginal CCS classification showed a reduction in ischemic segments of  $28\% \pm 4\%$  and patients with improvement had a slightly higher reduction of  $43\% \pm 6\%$  ( $P=0.06$ ). Interestingly, 1 of the 2 patients in the VEGF group without reduction of anginal complaints showed a  $>50\%$  reduction in ischemic segments. On the other hand, in the 7 patients in the DMR group reporting a reduction of anginal complaints, the reduction in ischemic segments ranged between 2% and 72%, and, in the 3 control patients, the reduction ranged between 28% and 64%. No significant relationships between quality-of-life parameters, such as RAND-36 and myocardial perfusion, were observed.

NOGA maps showed comparable voltage values (control,  $12.8 \pm 3.0$  mV; DMR,  $12.2 \pm 2.5$  mV; VEGF,  $12.5 \pm 3.5$  mV) as well as linear local shortening values ( $10.4\% \pm 4.2\%$ ,  $9.6\% \pm 3.8\%$ , and  $12.0\% \pm 3.3\%$ , respectively).

## Discussion

This study shows that with PET perfusion scanning, the effect of putative proangiogenic therapies can be compared. The implementation of this diagnostic modality demonstrated that only the hyperemic flow in the ischemic region of the VEGF group was significantly improved. PET scanning enabled us to compare myocardial perfusion changes associated with percutaneous catheter-based VEGF gene therapy, endomyocardial laser treatment, and optimal drug treatment in patients with end-stage CAD. Previous preclinical studies investigated the NOGA-catheter platform technique for percutaneous intramyocardial delivery of genes or laser energy<sup>9,21</sup>. The data from this study support previous clinical results<sup>9,21</sup> and confirm the safety and feasibility of this injection catheter technique.

The visualization of myocardial perfusion alterations in patients with refractory angina pectoris is important for the evaluation of proangiogenic treatment strategies. Transmyocardial laser revascularization has been reported to yield improved clinical outcomes with surgically based procedures as well as percutaneous approaches. Even though a reduction in anginal complaints was present in all of these studies, no consistent reduction in ischemia as assessed by radionuclide imaging was found<sup>22–25</sup>. It has been shown previously by PET that transmyocardial laser revascularization does not improve myocardial perfusion in the myocardial areas treated with laser therapy<sup>26</sup>. Despite the negative result on myocardial perfusion, a beneficial effect on the clinical condition was observed. In the present study, PET confirmed that direct myocardial laser revascularization exerted no visible effect on perfusion dynamics.

In contrast, the results of VEGF plasmid-based gene therapy trials have shown at least a comparable improvement in anginal complaints and a concomitant beneficial reduction of ischemia by SPECT imaging<sup>5–8</sup>. The present results may support these data, as the increased hyperemic perfusion (without change in rest perfusion) suggests a higher perfusion capacity.

An advantage of the PET technique over the SPECT technique is the lack of attenuation artifacts. With SPECT, irreversible defects are primarily caused by breast attenuation in the anterior wall and in the inferior wall by diaphragmatic attenuation<sup>27–29</sup>. SPECT may lead to misinterpretation, because attenuation artifacts will not disappear even if perfusion has really improved, such as in the case of truly proangiogenic treatment strategies. Furthermore, absolute quantification of myocardial perfusion with PET is a more accurate method in follow-up assessment of angiogenic therapies.

Interpretation of the findings in our study should involve consideration of the relative small number of patients included and that the study design was not a double-blind randomized setup to compare DMR with VEGF gene therapy. Furthermore, against the

background of the results of the DIRECT Trial and the Euroinject One Trial<sup>30,31</sup>, placebo effects must be accounted for. In the DIRECT Trial 2, doses of endomyocardial laser were compared with a sham procedure. The exercise time (primary endpoint) increased by 27 s, and 35 s in the high- and low-dose laser group versus 30 s in the sham group<sup>30</sup>. In the Euroinject One Trial, VEGF165 gene therapy was compared with control injections with empty plasmid. In this trial, at 3 mo, researchers were able to get readable SPECT images from 74 patients. Patients in the gene therapy group had an approximately 45% improvement in myocardial perfusion compared with about 30% in the placebo group, but this effect was not statistically significant ( $P = 0.18$ ).

The Euroinject One Trial results indicate that the issue of the right control group is important. In the control group, treated with empty plasmid, a considerable improvement was also found<sup>31</sup>. Possible proangiogenic properties of plasmid DNA as such may explain this observation<sup>32</sup>. Whether this also accounts for some of the proangiogenic effects of the active plasmid remains unclear. However, it is known that the plasmid can enter myocardial cells and produce measurable amounts of VEGF protein that can be found at locations distant from the injection site<sup>4</sup>.

## Conclusion

The application of VEGF gene versus endomyocardial laser therapy using comparable catheter techniques in subjects with refractory angina pectoris shows more beneficial effects in the VEGF group. Although the most appropriate control group in our opinion would be placebo, the present results add value to the available clinical evidence that VEGF gene therapy, in contrast with DMR, reduces myocardial ischemia. However, the data should be confirmed by a large double-blind, randomized, placebo-controlled trial.

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