The combined use of transmyocardial laser revascularisation and intramyocardial injection of bone-marrow derived stem cells in patients with end-stage coronary artery disease: one year follow-up

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Abstract

Background: There are a growing number of patients with end-stage coronary artery disease (CAD) and refractory angina. Angiogenesis may be induced by intramyocardial injection of autologous bone marrow stem cells, intensified by inflammation around channels performed by laser.

Aim: To assess the effect of a combined treatment consisting of transmyocardial laser revascularisation (TLMR) and intramyocardial injection of bone-marrow derived stem cells (bone marrow laser revascularisation, BMLR) in patients with refractory angina one year after the procedure.

Methods: Five male patients (age 49–78 years) with end-stage diffuse CAD, severe angina (CCS III/IV) despite intensive medical therapy and disqualified from prior coronary artery bypass grafting (CABG) or percutaneous coronary intervention were included. After heart exposure, at sites where CABG was impossible, TMLR was performed with the Holmium: YAG laser combined with injection of 1 mL of bone marrow concentrate into the border zone of a laser channel using a Phoenix handpiece.

Results: No deaths in the follow-up period were observed. All patients were in I CCS Class. One year after the procedure, left ventricular (LV) segments treated by BMLR tended to demonstrate stronger myocardial thickening compared to baseline (53.0 ± 7.5% vs. 45.0 ± 9.5%; p = 0.06). Using late gadolinium-enhanced imaging, new myocardial infarction was found after one year only in one LV segment treated by BMLR. The BMLR treated regions in the remaining subjects, as well as regions subtended by left internal thoracic artery in two subjects, did not show new myocardial infarction areas. In contrast, all subjects who underwent only BMLR procedure revealed new and/or more extensive myocardial infarct in regions not treated by BMLR.

Conclusions: Intramyocardial delivery of bone marrow stem-cells together with laser therapy is a safe procedure, with improvement in quality of life during follow-up. One year after the procedure, myocardial regions where BMLR was performed tended to demonstrate stronger myocardial thickening observed in cardiac magnetic resonance imaging.

Key words: stem cell, bone marrow, medical laser, angiogenesis, coronary artery disease

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INTRODUCTION

Coronary artery disease (CAD) remains the leading cause of death and disability in the European Union. The available options for treating CAD include lifestyle changes in conjunction with drug therapy, percutaneous coronary intervention (PCI) and coronary artery bypass graft surgery (CABG). The objective of each of these approaches is to improve myocardial perfusion of the heart and prevent the complications related to myocardial ischaemia.

Increasing numbers of patients who are not candidates for CABG and PCI continue to have severe angina despite maximal medical therapy [1]. It is estimated that 15–25% of patients undergoing CABG will have one or more major target areas incompletely revascularised due to diffuse CAD [2]. Incomplete revascularisation has been recognised by many authors as an independent predictor of perioperative mortality, particularly in the elderly [3, 4], and is associated with a lower freedom from cardiac death, acute myocardial infarction (MI) and cardiac events during long-term follow-up [5].

In multiple prospective randomised trials, transmyocardial laser revascularisation (TMLR) has been demonstrated as the only therapy that results in a significant improvement in angina and event-free survival and a reduction in cardiac hospitalisations compared to patients randomised to maximal medical therapy alone [6–9]. Long-term follow-up of TMLR as a primary therapy shows an enduring benefit over time and improvement in survival in the TMLR treated patients [10, 11]. The Society of Thoracic Surgeons and the International Society of Minimally Invasive Cardiothoracic Surgeons support the use of CABG plus TMLR when one cannot perform complete revascularisation [12, 13]. Operative mortality in patients with diffuse CAD after TMLR + CABG has been shown to be 1.5% vs. 7.6% after coronary bypass alone [14].

Tran et al. [15] have demonstrated an improvement in venous bypass flow in patients treated with TMLR after off-pump coronary artery bypass.

In another second small randomised trial of high risk CABG patients, the mortality benefit from CABG plus TAMR compared to CABG alone approached statistical significance [16].

Although TMLR’s superiority over medical therapy has been demonstrated, its effectiveness is not 100%. In one year, in up to 25% of patients treated with sole therapy TMLR, relief of angina symptoms was not significantly improved [6]. The potential synergy of combining TMLR with a biological therapy has been evaluated to increase TMLR’s efficacy. In a randomised, controlled clinical study on bone marrow cells therapy in patients after MI (REAPIR-AMI), a 2.5% increase in left ventricular ejection fraction (LVEF) was observed [17].

The important functional benefit of bone marrow cells may be mediated not through their autocrine effect but rather their paracrine effect. Survival of injured cardiomyocytes through the mobilisation of progenitor cells and the stimulation of angiogenesis may be promoted by paracrine secretion of growth factors and/or cytokines by bone marrow cells [18].

The above data suggests that bone marrow stem cells, placed in the proper environment, stimulate angiogenesis and play a role in the revascularisation of the ischaemic myocardium.

This study was to evaluate perioperative safety of this new hybrid therapy, performed in our Institution, as one of the first in Europe. Another goal of this study was to determine whether a new hybrid therapy: bone marrow laser revascularisation (BMLR) which combines TMLR plus stem cells with or without CABG improves segmental myocardial contractile function of heart muscle, quality of life (QOL) and ventricular function in patients who would be incompletely revascularised.

METHODS

Study group

Five patients with end-stage CAD, severe angina despite maximal medical therapy and disqualified from other intervention alone (CABG or PCI) were selected.

Informed consent was obtained from all patients. The study was approved by the local ethical committee (KBET/55/B/2007).

Inclusion criteria: age > 18 years, end-stage CAD, disqualified from direct revascularisation, CCS III or IV, patient on maximal medical therapy, LVEF > 35%.

All patients underwent preoperative electrocardiography (ECG), echocardiography, cardiac magnetic resonance imaging (MRI) and standard haematologic laboratory tests. All patients had maximal medical therapy and were disqualified from surgical or percutaneous revascularisation.

Baseline data for all the patients are given in Table 1. The mean time of the whole procedure in our group of patients was 155 min. The mean number of channels performed using

| Table 1. Demographic and clinical characteristics of study patients (n = 5) |
|-----------------------------|-----------------------------|
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| Age [years] | 67 ± 10 |
| Men | 5 |
| Previous condition: | | |
| Previous MI | 5 |
| Previous CABG | 2 |
| Previous PCI | 3 |
| Diabetes mellitus | 3 |
| Systemic hypertension | 5 |
| Hypercholesterolaemia | 4 |
| Peripheral vascular disease | 2 |
| Ejection fraction [%] | 48 ± 13 |
| CCS IV | 5 |

MI — myocardial infarction; CABG — coronary artery bypass grafting; PCI — percutaneous coronary intervention; CCS — Canadian Cardiovascular Society
The combined use of TLMR and intramyocardial injection of bone-marrow derived stem cells in patients with CAD

Table 2. Perioperative characteristics of patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Surgical procedure</th>
<th>Number of channels</th>
<th>Number of CD34</th>
<th>Off-pump</th>
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<tbody>
<tr>
<td>AM</td>
<td>BMLR</td>
<td>24 × antero-lateral</td>
<td>6.17 × 10⁶</td>
<td>Yes</td>
</tr>
<tr>
<td>LK</td>
<td>BMLR</td>
<td>13 × posterior, 13 × antero-lateral</td>
<td>16.75 × 10⁶</td>
<td>Yes</td>
</tr>
<tr>
<td>SS</td>
<td>BMLR</td>
<td>16 × antero-lateral</td>
<td>4.88 × 10⁶</td>
<td>Yes</td>
</tr>
<tr>
<td>WW</td>
<td>BMLR + LAD (LIMA)</td>
<td>24 × inferior</td>
<td>13.4 × 10⁶</td>
<td>No</td>
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<tr>
<td>MP</td>
<td>BMLR + LAD (LIMA)</td>
<td>22 × posterior</td>
<td>10.08 × 10⁶</td>
<td>Yes</td>
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BMLR — bone marrow laser revascularisation; LAD — left anterior descending artery; LIMA — left internal thoracic artery; CD34 — cell with surface antigen formerly known as haemopoetic progenitor cell antigen 1 (cluster of differentiation molecule)

A laser was 23 per patient (Table 2) and the average amount of CD34+ was 10.42 × 10⁶ cells.

CABG

After heart exposure, the surgeon made the final decision as to whether any of the narrowed coronary arteries was suitable for bypass grafting. In two out of five cases, CABG was performed on coronary arteries suitable for revascularisation (diameter of coronary artery was suitable and calcification of the coronary vessels wall allowed grafts to be performed). Patients MP and WW had left internal thoracic artery implanted to left anterior descending artery. In one case (MP), surgery was performed without cardiopulmonary bypass (CPB), and in patient WW we used CPB and blood cardioplegia.

Myocardial infarction was reported based on the Universal Definition of Myocardial Infarction [19] — biomarker values more than five times the 99th percentile of the normal reference range during the first 72 h following CABG, when associated with the appearance of new pathological Q-waves or new left bundle branch block, or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium.

The patients were transferred to a postoperative intensive care unit for a mean one day and then moved to a postoperative unit. Patients were discharged from hospital 7–15 days after the procedure.

Clinical evaluation of patients’ symptoms was performed three, six and 12 months after the operation. Quality of life was estimated using the EuroQol (EQ-5D) protocol.

Bone marrow laser revascularisation

Autologous bone marrow stem cells were harvested from the pelvic bone during the same general anaesthesia. Usually, from one patient we took 200 mL of bone marrow aspirate and concentrated this volume to 30 mL of bone marrow stem cells concentrate using the Harvest BMAC System. The Harvest BMAC System processes autologous bone marrow in approximately 15 min and has been documented to provide high bone marrow derived stem cell concentrations. The system maintains the stem cells in their native environment from the time of harvest, with minimal handling, through to implantation into the patient.

Two patients had laser revascularisation and stem cells concentrate injection through sternotomy. In one case, a patient after CABG with severe diffuse CAD verified in coronary arteries angiography (i.e. no chance of direct revascularisation), we decided to perform left small thoracotomy for the BMLR procedure. Both accesses (sternotomy or left small thoracotomy) are adequate for the BMLR procedure, and in selected cases can be performed through a smaller incision.

The BMLR procedure was performed with Holyum: YAG Medical Laser Eclipse 2000 (Cardiogenesis) and the Phoenix combination delivery system. The Phoenix handpiece consists of a laser fibreoptic in conjunction with a three needle biologic delivery module that allows the precise placement of stem cells into the border zone created by the laser channel.

In areas without revascularisation, BMLR was performed according to this rule: one channel every square centimetre, avoiding the coronary vessels. Additionally, in the border zone, 1 mL of bone marrow stem cells concentrate was injected per channel, using the Phoenix handpiece.

Cardiac magnetic resonance: imaging protocol

Breath-hold, ECG gated cardiac magnetic resonance (CMR) imaging was performed on a 1.5 T whole-body scanner (Magnetom Sonata Maestro Class, Erlangen, Germany) equipped with a dedicated cardiac software and six element phased array coil. After scout imaging, cine and late gadolinium-enhanced (LGE) imaging was performed, comprising LV short-axis as well as two-chamber, four-chamber and long axis apical views. Cine imaging was obtained using balanced steady-state free precession gradient echo (slice/gap thickness = 8/0 mm, matrix = 256 × 192, in-plane resolution = 1.3 × 1.3 mm², TR/TE = 39/1.1 ms, flip angle = 590). LGE imaging was carried out 10 min after i.v. infusion of 0.15 mmol/kg body weight gadobutrol (Gadovist, Bayer Schering Pharma, Berlin, Germany), using a T1-weighted segmented inversion-recovery pulse sequence (slice/gap thickness = 8/0 mm, matrix = 256 × 192, in-plane resolution = 1.3 × 1.3 mm², TR/TE = 650/4.9 ms, flip angle = 300, TI to null normal myocardium).
**Cardiac magnetic resonance: image analysis**

Cine and LGE images were assessed off line by two independent observers blinded to clinical data using dedicated software (QMASS Medis, Leiden, the Netherlands). In the presence of a discrepancy in qualitative assessment, a consensus between readers was reached.

**Cine images.** Left ventricle short-axis as well as two-chamber, four-chamber and long axis apical views were used for segmental wall motion analysis. A 17 segment model of LV was used for analysis. Segmental myocardial contractile function was assessed in terms of endocardial motion and/or systolic wall thickening and scored as: 1 — normal, 2 — hypokinetic, 3 — akinetic, 4 — dyskinetic. Wall motion score index was calculated as the summation of individual segmental scores divided by the total number of LV segments. Endocardial and epicardial borders were outlined as previously described on short axis images to calculate LV end-diastolic volume, end-systolic volume, myocardial mass and EF [20]. The mean end-diastolic wall thickness, end-systolic wall thickness and myocardial thickening were derived for each LV wall.

**LGE images.** LGE images were assessed qualitatively for the presence and location of hyperintense lesions in contrast to hypointense viable myocardium. The number of affected segments was calculated. The extent of contrast enhancement was planimetrered on the short axis images using an image intensity level $\geq 5$ SD above the mean of remote myocardium to define contrast enhancement. Transmural extent of infarction (TEI) was scored as 0 — no infarction, 1 — 1–25%, 2 — 26–50%, 3 — 51–75%, and 4 — 76–100% of LV wall thickness, respectively.

**Statistical analysis**

Continuous variables are presented as means ± standard deviation (SD) or medians as appropriate. Categorical variables are presented as percentages. Continuous variables were compared using the t-test where appropriate. A p value of less than 0.05 was considered to be statistically significant. Analyses were performed with Statistica ver. 7.1 (Stat Soft Inc.).

**RESULTS**

No perioperative complications occurred in our group. Troponin I (our laboratory norm $\leq 0.5$ ng/mL) was evaluated three times after surgery (0, 4 and 8 h on intensive care unit) and was similar in all patients (BMLR vs. BMLR+CABG: 5.5 vs. 10.3 ng/mL; 7.4 vs. 6.5 ng/mL; 10.9 vs. 10.5 ng/mL), except one patient (WW: BMLR+CABG). No other symptoms of ischaemia were observed. In one case, we considered temporary postoperative ischaemia.

We observed malignant ventricular arrhythmias in no cases. No mortality or other major cardiac events were observed during hospitalisation. All patients were discharged from hospital in general good condition with no chest pain.

During one year follow-up, no major advanced cardiac events were observed and mean LVEF was 46% (50% — mean LVEF from MRI). The symptoms of angina decreased at least two classes according to the CCS classification. Based on the EuroQol test, in all cases QOL improved. Betterment of everyday activity from 8.5 to 6.0 points after surgery was observed (Table 3). Four patients did not feel any discomfort from CAD and five declared satisfaction from results of surgery.

CMR results are displayed in Table 4. One year after the procedure, LV segments treated by BMLR tended to demonstrate stronger myocardial thickening compared to baseline (53.0 ± 7.5% vs. 45.0 ± 9.5%; p = 0.06). Only one patient (AM) showed a decrease in myocardial thickening in BMLR region (Fig. 1). One year after the procedure, new or more severe segmental abnormalities were present in two subjects (AM, SS). The abnormalities were found both in BMLR (AM) and remote regions of BMLR (SS). No other symptoms of ischaemia were observed in two subjects with new MI in regions not treated by BMLR.
DISCUSSION

BMLR is an advanced combination therapy performed by cardiothoracic surgeons on a beating or non-beating heart, in which a laser device is used to create transmural channels in target ischaemic heart muscle and deliver autologous bone marrow aspirate concentrate to the tissue surrounding each of the laser channels. TMLR and bone marrow derived stem cell therapy have each demonstrated the ability to generate angiogenesis and to improve clinical outcomes in cardiac applications. Using the laser to create

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*Enlarged myocardial infarction area compared to baseline examination; E — enrollment; F-up — follow-up, WMSI — wall motion score index; TEI — transmural extent of infarction; #WMA — number of left ventricular segments with wall motion abnormalities; A — akinetic, H — hypokinetic; LIMA — left internal thoracic artery; LAD — left anterior descending artery; LV — left ventricle, EF — ejection fraction; BMLR — bone marrow laser revascularisation; LGE — late gadolinium-enhanced; SV — systolic volume
The major findings of our study in the follow-up period were a reduction of angina symptoms, improvement in QOL, and positive changes observed in CMR imaging.

Symptoms of coronary heart disease are a major burden for patients, negatively affecting their daily activities. Treatment of patients with CAD is focused on the one hand to treat the disease, but also to reduce the symptoms of angina. In our patients, after a year of observation, we noticed improved everyday activity and QOL. A similar observation with reduced clinical symptoms of CAD after bone marrow stem cells and laser revascularisation procedures using the same device have been reported by Reyes et al. [21].

MRI allows the objective evaluation of segmental myocardial contractile function and MI areas after revascularisation performed by medical laser and implantation of stem cells. LV walls treated by BMLR tended to demonstrate stronger myocardial thickening compared to baseline. New MI was found after one year only in one LV segment treated by BMLR (LK). The BMLR treated regions in the remaining subjects as well as regions subtended by LIMA in two subjects did not show new MI areas.

**Limitations of the study**

The main limitation of the study was the small number of patients. Based on our own observations, BMLR treatments should be conducted only in areas with preserved viability (not within the scar), after having a thorough analysis of LV with MRI.

Based on the results of our pilot study, BMLR is a safe new hybrid therapy, with no major complications during the surgery or follow-up and improves QOL in patients with end stage CAD. It should be emphasised that all patients were disqualified from direct revascularisation (CABG or PCI); however, using a new surgical technique (BMLR) patients can be re-qualified and treated with clinical improvement (a reduction of two classes on the CCS scale) and decreased pharmacological treatment. Particular benefit was observed in patients with the hybrid procedure (BMLR+CABG). However, a reduction in angina symptoms and an improvement in QOL were observed in both groups.

The growing population of patients with end-stage CAD requires us to explore additional treatment options to provide improved benefits to this high-risk group of patients.

**CONCLUSIONS**

BMLR is a safe new hybrid therapy, with no major complications during the surgery. After one year, the symptoms of angina decreased by at least two classes according to the CCS classification. Based on the EuroQol test, in all cases QOL improved. One year after the procedure, LV segments treated by BMLR tended to demonstrate stronger myocardial thickening compared to baseline. New MI was found after one year only in one LV segment treated by BMLR (LK). The

![Figure 1. Change in mean end-diastolic wall thickness (A), end-systolic wall thickness (B) as well as myocardial thickening in individual left ventricular walls (C), which underwent BMLR therapy](image)
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BMLR treated regions in the remaining subjects, as well as regions subtended by LIMA in two subjects, did not show new MI areas.

Acknowledgments
We thank Cardiogenesis Company for providing the Phoenix handpiece, Harvest Company for providing the Harvest BMAC System, and M. Nawiesniak for intraoperative photography. We are also indebted to Keith B. Allen and Anetta Undas for their valuable comments.

Conflict of interest: none declared

References
Zastosowanie lasera medycznego
i komórek macierzystych w leczeniu krańcowej postaci choroby niedokrwiennego serca:
roczna obserwacja

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Streszczenie

Wstęp: Populacja pacjentów z krańcową, rozsianą postacią choroby niedokrwiennego serca (ChNS), którzy nie mogą być optymalnie leczeni metodami klasycznymi [pomostowanie aortalno-wieńcowe (CABG), przeszkołne interwencje wieńcowe (PCI) oraz maksymalna terapia lekowa] rośnie, stanowiąc duże wyzwanie w codziennej praktyce klinicznej. Wykonanie kanałów w mięśniu sercowym, ze względu na wytworzone miejscowo środowisko zapalne, może nasilać tworzenie nowych naczyń (neoangiogenezę). Wszczepienie komórek macierzystych dokładnie w bezpośrednie otoczenie (border zone) wytworzonej przez laser kanałów (BMLR, bone marrow laser revascularisation) może nasilić ich różnicowanie w kierunku neoangiogenezy, poprzez stymulowanie tego procesu w wyniku lokalnego środowiska zapalnego. W klinice, w której pracują autorzy niniejszej pracy, jako w jednej z pierwszych w Europie, zaczęto stosować tę metodę u wybranych pacjentów z ChNS.

Cel: Celem badania była ocena jakości życia, dolegliwości wieńcowych i wyników rezonansu magnetycznego (MRI) po zastosowaniu lasera medycznego z równoczesną implantacją komórek macierzystych (BMLR) po roku od zabiegu.

Metody: Do badania włączono 5 pacjentów, w wieku 49–78 lat, z rozsianą postacią ChNS, objawami klinicznymi (CCS III/IV) mimo optymalnej terapii lekowej i niekwalifikujących się do klasycznych CABG i PCI. W pierwszym etapie operacji z talerza kostki biodrowej pobierano komórki macierzyste i poddawano je procesowi zagęszczania za pomocą Harvest BMAC System. Po ok. 20 min uzyskano 20–30 ml świeżej, zagęszczonej zawiesziny zawierającej duże stężenie komórek macierzystych. U wszystkich pacjentów, po uzyskaniu dostępu do serca (sternotomia lub torakotomia), oceniano jeszcze raz naczynia wieńcowe i w razie możliwości wykonano CABG. W obszarach mięśnia sercowego, w których nie zrobiono CABG, przeprowadzono laserową rewaskularyzację z użyciem laseru Holmium: YAG (śr. 23 kanały na pacjenta) oraz implantowano komórki macierzyste za pomocą systemu Phoenix (śr. liczba CD34+ to 10,42 × 10⁶). Przed zabiegiem i rok po nim wykonano MRI z użyciem specjalnego oprogramowania przeznaczonego do oceny mięśnia sercowego.

Wyniki: Nie zaobserwowano żadnych powikłań okołooperacyjnych. Wszyscy pacjenci zostali wypisani ze szpitala w stanie ogólnym dobrym. W okresie obserwacji (1 rok po operacji) nie zanotowano zgonu, udaru ani klinicznie potwierdzonego zawału serca. Objawy dławicy pierśmiowej zmniejszyły się o co najmniej dwie klasy wg klasyfikacji CCS. O ile pacjenci byli w stanie wykonywać codzienne czynności bez bólu w klatce piersiowej i zadeklarowali znaczącą poprawę jakości życia. Rok po operacji u osób leczonych BMLR w MRI wykazano poprawę grubienia miokardium w porównaniu z badaniem przedoperacyjnym (53,0 ± 7,5% vs. 45,0 ± 9,5%; p = 0,06). Nowy obszar zawału stwierdzono tylko w 1 segmencie lewej komory u pacjenta poddanego BMLR. U pozostałych osób w obszarach poddanych BMLR i w obszarach po pomostowaniu LAD-LIMA (2 pacjentów) nie wykazano nowego obszaru zawału. Natomiast w obszarach niepoddanych BMLR i pomostowaniu stwierdzono nowy obszar zawału lub powiększenie się starego obszaru zawału.

Wnioski: Na podstawie wyników tego pilotażowego badania można stwierdzić, że BMLR jest bezpieczną metodą, obarczoną niewielkim ryzykiem poważnych powikłań. W rocznej obserwacji zanotowano poprawę jakości życia i zmniejszenie dolegliwości wieńcowych. W MRI wykazano zwiększone grubienie mięśnia lewej komory w obszarach poddanych BMLR. Ostateczna ocena skuteczności i bezpieczeństwa zastosowania BMLR wymaga przeprowadzenia dalszych badań obejmujących większą grupę pacjentów.

Słowa kluczowe: komórki macierzyste, szpik kostny, laser medyczny, angiogeneza, choroba niedokrwienna serca

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