Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006;355:1199-209.

SUPPLEMENTAL INFORMATION

Cell harvest and preparation

Bone marrow (50 ml in 10.000 IU heparin) was obtained from the iliac crest in local anesthesia four to seven days after PCI. Upon receipt in the cell processing laboratory, the aspirate was diluted in 75 ml 0.9 % saline. The diluted bone marrow aspirate was layered on top of Isopaque – Ficoll (Lymphoprep™, Axis-Shield, Norway) and subjected to centrifugation at 800xG for 20 minutes. The mBMC were washed three times using 0.9 % saline containing 5 % autologous heparin-plasma and centrifugation at 250 x G for 10 minutes prior to final resuspension in 11 ml 0.9 % saline with 20 % heparin-plasma. A small sample of the mBMC suspensions intended for use the next day was collected in a pediatric blood culture flask for bacteriological testing, the rest was stored at 6 ºC. Before intracoronary injection, the mBMC suspension was filtered through

a 70 µm strainer, a small sample was taken for bacteriology testing and assessment of cell yield, viability, aggregate formation and content of stem cell subsets, leaving approximately 10 ml for the patient. Pre-specified cell quality criteria were: negative bacterial specimens, viability ≥90 %, and aggregation (clumps of ≥ 3 cells) involving < 10 % of all cells. Cell preparations were performed in a good manufacturing practice-regulated cell processing laboratory.

Cell subsets and quality control

The mean volume of aspirated bone marrow was 49±9 ml. Cells were counted and tested for viability in a Kova Gasstick slide (Hycor Biomedical Inc., Garden Grove, CA) using an acridin orange/ethidium bromide dye exclusion assay under fluorescent light. Median cell viability was 95 % (interquartile range, 94 to 97). Median number of injected viable mBMC was $68x10^6$ (interquartile range, $54x10^6$ to $130x10^6$). The median number of CD34+ cells (anti-CD34/PE combined with BD Trucount, analysed on a FACSort cell analysis instrument, BD Biosciences, San Jose, CA) in the mBMC sample was $0.7x10^6$ cells (interquartile range, $0.4x10^6$ to $1.6x10^6$ cells). In a subgroup of 23 patients, the median number of CD34+CD133+ cells (anti-CD34/FITC, Diatec, Oslo, Norway and anti-CD133 APC, Miltenyi Biotech, Bergisch Gladbach, Germany) was $0.8x10^6$, while the median total number of CD133+ cells (CD34+CD133+ plus CD34-CD133+ cells) was $1.7x10^6$.

Cell transfer

Cell injections were performed with a standard PCI technique. After administration of heparin 100 IU/kg body weight, the ostium of the left coronary artery was intubated with a 6 French guiding catheter and a 0.5 mm oversized over-the-wire balloon catheter was advanced to the proximal part of the stent. The balloon was inflated with low pressures (< 2 bar) during distal injection of one third (approximately 3.3 ml) of the mBMC suspension and 90 seconds of no-flow. In total, there were three injections with five minutes of re-flow between balloon inflations. ECG with standard leads was monitored during the procedure and any chest pain and/or arrhythmias were registered. After the procedure, ECG was monitored for at least 24 hours. Creatine kinase MB, troponins and 12-lead ECG were obtained before and the day after the procedure.

Myocardial scintigraphy

Perfusion imaging was performed as ECG-gated SPECT after injection of 99mTc-tetrofosmine (Myoview™, Amersham Health, UK). The patients met fasting. At baseline, there were no medication restrictions. At six months beta-blockers, calcium antagonists and nitrates had been withheld for 24 hours. The injected dose was 500MBq in baseline studies, and 250-300MBq (weight-adjusted) in 6 month studies. An Exeleris (GE Medical Systems) processing station, with the 4D-MSPECT™ software, was used for processing of all recordings and assessment left ventricular volumes and infarct size (proportion perfusion defect). Two nuclear medicine technicians unaware of treatment allocation processed all recordings. For each patient, baseline and six month recordings were processed by the same technician. Technician A processed 53, and technician B

processed 47 pairs of patient recordings. Intra-observer variability for LVEF was assessed in a random subset of 25 six months recordings. The intraclass correlation coefficient was 0.95 (95 % confidence interval 0.88 to 0.98).

Echocardiography

Echocardiograms were recorded using a Vivid 7 scanner (GE Vingmed Ultrasound, Horten, Norway). Three consecutive cineloops of three apical views were recorded for analysis of left ventricular volumes. Care was taken to avoid foreshortening of the left ventricle long axis. Images were digitally stored, blinded and analyzed on a separate work station by a single physician. EchoPAC PC software (GE Vingmed Ultrasound) was used. Left ventricular volumes were computed using the modified Simpson`s rule according to current guidelines[18]. Due to better reproducibility, the apical long-axis view was preferred to the two-chamber view in combination with the four-chamber view unless the latter was of clearly better quality[19]. The cineloop with best endocardial borders was chosen, and the contour of the left ventricular cavity was manually traced in end-diastole and end-systole. Intra-observer variability for LVEF was assessed in a random subset of 25 six months recordings. The intraclass correlation coefficient was 0.92 (95 % confidence interval 0.83 to 0.96).

Magnetic resonance imaging

MRI was performed using a 1.5 tesla Siemens scanner (Magnetom Vision Plus for the first 18 months of the study, and Magnetom Sonata, Siemens, Erlangen, Germany, for the last 10 months), and a phased array body coil. Breath-hold cine images in two-chamber,

four-chamber and short axis views were acquired with one of two ECG-triggered segmented gradient echo techniques, FLASH or trueFISP (the last available with Magnetom Sonata only). Each patient was examined with the same cine technique at baseline and at six months follow-up (n=63 pairs for FLASH, n=24 pairs for trueFISP). Calculation of left ventricular volumes was based on the biplane area-length method[20, 21]. Approximately 10 – 20 minutes after intravenous injection of 0.2 mmol/kg gadopentetate dimeglumine (Magnevist, Schering, Berlin, Germany), late contrast enhancement images were obtained, first in two-chamber and four-chamber views, and then with multiple short axis slices covering the entire left ventricle with a slice thickness of 7 mm and an interslice gap of 3 mm. A breath-hold segmented magnetization-prepared turbo gradient echo sequence was used with an inversion time of 210 – 260 ms. For each slice the total area of the left ventricular wall and the area of late enhancement were manually drawn, and the areas were summed for calculation of total left ventricular wall volume and total late enhancement volume . Infarct size is presented as total late enhancement volume and as a proportion ((total late enhancement volume / total left ventricular wall volume) x 100 %). Analyses were performed by two physicians unaware of treatment allocation. Inter-observer variability for LVEF was assessed in two random subsets of 25 patients at six months for FLASH and trueFISP, respectively. For FLASH, the intraclass correlation coefficient was 0.85 (95 % confidence interval 0.67 to 0.93. For trueFISP, the intraclass correlation coefficient was 0.98 (95 % confidence interval 0.96 to 0.99).