Transepicardial With Transseptal Autologous CD 133+ Bone Marrow Cell Implantation in Patient Following CABG Surgery

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Study Protocol

Objectives

Numerous publications in the last decade have been conducted to study the effect of stem cell therapy in improving cardiac function, but the results and the type of cell and implantation procedure vary considerably. The CD133+ bone marrow cell has been shown to possess a high angiogenesis capacity. One previous study published by Nasseri et al. reveals that trans-epicardial implantation of CD133+ for low LVEF patients who underwent CABG showed no significant improvement; nevertheless, it is stated that this result might differ if the trans-septal region is included as a site of stem cell implantation. Therefore, we perform a randomized clinical trial at our Department of Cardiovascular Surgery of National Cardiovascular Center Harapan Kita, Indonesia, and include additional trans-septal implantation of CD133+ to assess the impact of stem cells in improving cardiac function.

Design and Methods

This study was conducted between March 2016 and June 2018, and the study had been approved by the Institutional Review Board and Ethics Committee of National Cardiovascular Centre Harapan Kita (No. LB.02.01/VII/086/KEP.007/2016) and Universitas Indonesia (No:999/UN2. F1/ETIK/XI/2016). This study had also been registered on ClinicalTrial.gov (**NCT02870933).** All patients enrolled in this study had been informed about the procedures and signed the written informed consent. Thirteen patients were randomized and assigned to

each group. Patients in the case group received CABG + implantation of CD133+, and the control group received a CABG-only procedure.

Inclusion criteria for this study were as follows: i) aged <70, three vessels with coronary artery disease, and indication for coronary artery bypass graft (CABG) surgery; ii) impaired resting global LVEF < 35% with hypoperfused myocardium and abnormal septal wall motion were assessed by cardiac MRI; iii) hypoperfused resting state of LV on MRI. Exclusion criteria were those with i) CAD with valvular disease requiring concomitant valve surgery; ii) acute myocardial infarction; iii) a contraindication for MRI or bone marrow cell (BMC) aspiration procedure; iv) a history of ventricular arrhythmia (≥ Lown III); v) coagulation disorders, including familial hemophilia, sign/symptoms of bleeding disorders, platelets < 80000 mL; vi) chronic obstructive pulmonary disease (COPD); vii) infectious diseases (HIV, Hepatitis B, and HCV); viii) abnormal liver function and renal function test; ix) ventricular conduction disorders; x) electrolyte imbalances; xi) history of immunosuppressant medication, cytotoxic agents, and radiotherapy within four months before date of surgery.

The minimum of 13 patients per group was required to show the inter-group difference in LVEF, with a power (b) of 80% at a two-sided a level of = 0.05. However, 15 patients in each group were included for dropout anticipation.

Bone Marrow (BM) Aspiration

Bone marrow aspiration was done 24 hours before the scheduled CABG**.** Patients were anesthetized locally, and aspiration was carried out from the posterior iliac crest. A specimen of 190–210 mL of BM volume was harvested and diluted with 25 mL of normal saline containing 40,000U heparin.

CD133+ Separation Process

The aspirate was then transferred to our stem cell facility. Phosphate buffer saline was added until a total volume of 450 mL was reached and then centrifuged to dispose of the platelet. 1.5 mL of Magnetic MicroBeads® anti-CD133 labeling (Miltenyi Biotec) were added and incubated at room temperature for 30 minutes. Separation of the CD133+ was conducted using the *CliniMACS*® Magnetic Separation Device (Miltenyi Biotec). The final product was stored at 4–8 ^{o}C. The final aspirate volume for implantation was 20 mL, with a CD133+ count ranging from 5–10 million cells. Ten-mL samples were drawn to assess cell numbers, proof of sterility, purity, and the viability of the CD133+ using flow-cytometry FACS Aria III (BD Biosciences). The volume of the initial BM was 190–210 mL. The purity of the CD133+ end-product was 84.4–90.1%, with the viability of 95.2–94.4%.

Surgery

Anesthesia and CABG surgery was carried out according to the regular surgical protocol. The CD133+ was prepared in 20 one-mL syringes with 25G needles (0.5 x 25mm). Trans-septal implantation of CD133+ was performed by going along the left anterior descending artery (LAD). The correct needle placement within the interventricular septum was assessed with the use of trans-oesophageal echocardiography. Each injection of 0.5 mL of CD133+ was administered at injection sites approximately 1.0 cm apart. Ten injections were administered in the trans-septal region, and the other 30 injections were injected along the border area of the hypo-kinetic/hypo-perfused segment, which had been identified by MRI or by visible scarring area and epicardial muscles discoloration.

Cardiac magnetic resonance imaging and analysis

All patients were assessed at the same imaging facility in our hospital before the surgery and six months after the procedure. Our imaging facility was equipped with a Philips Achieva 1.5T

MRI. All subjects underwent resting and stress (adenosine 140 μg/kg/min) MRI with contrast agent *gadopentate dimeglumine* (0.05 mmol/kg). Left ventricular ejection fraction (LVEF), scar size, and wall motion score index (WMSI) were analyzed using *software* cvi42®.

Quality of life and aerobic capacity

The Minnesota Living with Heart Failure Questionnaire (MLHFQ) and the six-minute walk test (6MWT) were used to evaluate the quality of life and the aerobic capacity before and six months after the procedure. Registered cardiologists assessed the 6MWT for all patients recruited. The Minnesota Living with Heart Failure Questionnaire (MLHFQ) consisted of 21 questions that were used to assess the quality of life.

Statistical Analysis Plan (SAP)

All statistical data were analyzed using *IBM SPSS Statistics version 21.0* (*SPSS Inc, Chicago, IL, USA*). Continuous data were presented as mean ± standard deviation or median and interquartile range; categorical data were presented as number (n) and percentage. Shapiro-Wilk's test was utilized to assess the distribution of the data. Data were analyzed with independent t-test or Mann Whitney test accordingly. The measurement of scar size proportion presented as improved and non-improved categorical data were compared by the Chi-square test. In all cases, *the P-value* of < 0.05 was considered statistically significant. All of our results were measured by comparing the pre- and post-intervention changes of both groups. Data on boxplot were presented in median and minimum-maximum. Especially, statistical analysis in changes LVEF variable and changes in WMSI used paired t test, analysed defect segment perfusion, MLHFQ and the 6MWT we used Mann-Whitney test and scar size proportion we used chi-square test.