Chapter 6

Stem Cells for Acute Myocardial Infarction: Safety and Efficacy

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A. Introduction

ST-segment elevation myocardial infarction (STEMI) is a leading cause of subsequent mortality worldwide. Rapid reperfusion of infarct-related arteries, either with thrombolytic therapy or primary percutaneous coronary intervention (PCI) with stent implantation, has been demonstrated to improve the prognosis of patients with STEMI significantly. However, fewer than 50% of patients with STEMI currently achieve adequate reperfusion before irreversible damage to the supplied myocardial tissue occurs. It is known that

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myocardial necrosis starts rapidly after coronary occlusion, usually before reperfusion can be achieved. Since adult cardiac myocytes cannot routinely re-enter the cell cycle and proliferate, the heart's capacity for self-repair to compensate for the massive loss of cardiac myocytes is limited. The loss of viable cardiac myocytes during STEMI subsequently initiates a process of adverse left ventricular (LV), characterized by mechanical expansion of the scarred, infarcted myocardium, followed by progressive LV dilatation and dysfunction, culminating in heart failure and death. Additionally, failed reperfusion can also lead to potential ischemia-reperfusion (I/R), which can further damage cardiac myocytes through ferroptosis activity within the cells due to increased iron accumulation during ischemic myocardium, leading to an increased myocardial oxidative stress and cell death (Song et al., 2020).

Furthermore, increased oxidative stress from I/R could induce high levels of Interleukin-18 (IL-18) and potentially aggravate inflammation and tissue damage, leading to more acute coronary syndromes, myocardial dysfunction, and eventual heart failure (Huang et al., 2020). Indeed, in chronic heart failure and after myocardial infarction, most of the compensatory gain in myocardial mass results from hypertrophy rather than cell division (Kalra et al., 2018). Various pharmacological agents have been demonstrated to reduce early mortality rates and the risk of further heart attacks. However, especially in the case of large areas of infarction, other treatments for the regeneration of damaged cells after STEMI are deemed necessary (Botleroo et al., 2021). Cell therapy has emerged as a promising alternative strategy for the regeneration of cardiac cells. The rationale for cell therapy to be administered after STEMI derives from the assumption that given the insufficient regeneration in the injured heart tissue, those cells are expected to be able to replace or repair damaged vascular and cardiac tissue, or even secrete exosomes targeting specific mechanisms causing myocardial cell damage and inhibiting those mechanisms to prevent further damage of the heart tissue (Fisher et al., 2015; Seitz et al., 2019).

B. Factors that Influence the Succes of Stem Cell Therapy

The success of stem cell therapy for clinical use remains to be validated and many issues must be elucidated. Those issues, among others, are selection of appropriate types and number of stem cells, routes of administration, assessment of response to cell therapy, survivability rate due to site environment stresses as well as regulatory, and ethical issues (Amiri et al., 2015; Satessa et al., 2015).

1. Source

Mesenchymal stem cells (MSCs) are a choice of cell therapy due to multipotent factors, survival ability, plasticity, engraftment, paracrine activity, and low immunogenic potential (in allogeneic use). The cellular mechanism of MSCs therapy in cardiac regeneration is still unclear, but is thought to be through several mechanisms, including inflammatory response and angiogenesis, antifibrotic, differentiation of stem cells into new cardiomyocytes, and autophagy processes. Many clinical trials have been conducted to investigate the efficacy of MSCs derived from various tissue types. MSCs can be isolated from bone marrow, adipose tissue, and umbilical cord. Adult allogeneic MSCs derived from bone marrow are the type most widely used in the treatment of cardiovascular disease. However, many researchers are turning to use mesenchymal stem cells from the umbilical cord (UC-MSC) because their advantages include being easy to obtain without invasive procedures, having a primitive phenotype, and having long telomeres so the potential for proliferation and differentiation is better. The study by Gao et al. (2015) reported that post-transplant UC-MSC clinical events were not significantly different compared to the placebo group but death after 18 months of follow-up was found in the placebo group, whereas in the UC-MSC group, there were no deaths, recurrence of myocardial infarction, thrombosis, cerebral infarction or arrhythmias. Another study conducted on humans reported that there was a decreased scar size, an improvement in tissue perfusion, and an

increase in regional function after being injected with mesenchymal stem cells via intramyocardially (Mahmud et al., 2022).

Some preclinical studies have demonstrated that UC-MSC expressed cardiac-specific molecules, enabling them to readily differentiate into cardiomyocyte-like and endothelial cells in vitro (Jung et al., 2017). Paracrine effects exerted by UC-MSC may also enhance vascular regeneration and cardiomyocyte protection, such as through the upregulation of various types of exosomes in the form of miRNAs, which was shown to inhibit cardiomyocyte apoptosis, reducing the fibrotic area, inhibiting ferroptosis and improving the cardiac function (Bartolucci et al., 2017; Huang et al., 2020; Song et al., 2020; Zhu et al., 2020). From a genetic standpoint, several studies state that MSCs maintain genetic stability throughout the entire in vitro culture phase. A study by Zamani et al. (2022) showed that in different passages of culture, there was no significant abnormality was observed in the karyotype and morphology of MSCs. Based on the explanation above and in attempting to enhance the effects of stem cell therapy, we choose to use allogeneic umbilical cord mesenchymal stem cells (UC-MSC) for this study. Allogeneic mesenchymal stem cell therapy is confidently secure for administration, fortified by a meticulous donor selection process and an abundance of research findings consistently reaffirming its safety. The allogeneic properties of MSCs offer the possibility of early administration as an "off-the-shelf" product and they can be subjected to higher quality control than autologous products (Crisostomo et al., 2015).

2. Route of Administration and Dose

The delivery method of stem cells also plays a crucial aspect in therapeutic efficacy. Intracoronary and intramyocardial routes are two routes that have been mainly used (Hénon, 2020). Based on the Transplantation of Progenitor Cells and Regenerative Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) study on 20 patients who were administered stem cells via intracoronary using a stop-flow

technique with an over-the-wire balloon, it was reported to be safe, without experiencing thrombotic complications, arrhythmias, or other cardiovascular events. A study by Zhang et al. (2021) used bone marrow MSCs via intracoronary and showed no adverse reactions, such as stent thrombosis, recurrence of myocardial infarction, arrhythmia, tumor, and myocardial fibrosis. In his study, an ultra-long guide wire was inserted, followed by the insertion of a balloon catheter distal to the stent. Then, the ultra-long guide wire is removed, and the balloon catheter is inflated with pressure until there is no blood flow to the distal target vessel (balloon inflation period). A study by Kim et al. (2018) provides bone marrow MSCs in patients with acute myocardial infarction via intracoronary. The results reported that there was no adverse event with significant improvement in LVEF 4-month and 12-month follow-up. Based on the explanation above, it can be concluded that intracoronary administration is feasible and safe. In this study, we used a dose of 50 million cells as previously reported experience with this number of large-size stem cells after STEMI has been safely reported for use (Bobi et al., 2017).

The intravenous route is also used for therapy, but only in a few trials and is most often used in experimental small-animal studies (Hénon, 2020). The intravenous route is the most convenient and least invasive. It can be used because of the preponderance of physiological homing signals of stem cells to migrate toward the site of injury. Strategies of intravenous infusion are particularly appropriate for recently infarcted as well as reperfused myocardium. The study of Charles et al. (2020) administering exosome MSCs intravenously to pigs showed a reduction in infarct size of ~30%–40% in the exosome group on day 28, with a value of infarct size (5.96 ± 0.99% for exosome group vs. 10, 23 ± 1.02% for control, $p < 0.01$). In a review article by Razeghian-Jahromi et al. (2021), doses of 0.5, 1.6, and 5 million cells/kg were shown to be safe for intravenous administration. Therefore, for intravenous administration in this study, we used a dose of 2×10^6 cells/kg.

3. Preparation of Stem Cells and Timing of Administration

The stem cells that are used for therapy must meet the requirements for clinical application. However, the lack of standardized methods for isolating, expanding, and validating stem cells presents significant obstacles and challenges to producing high-quality stem cells.

For this study, the stem cells were provided by PT. Prodia StemCell Indonesia and the Ethics Committee of the Faculty of Medicine, University of Indonesia (No. 245/UN2.F1/ETIK/PPM.00.02/2019), with regards to the protection of human rights and welfare in medical research has reviewed and approved the research protocol and information of the potential patients. The baby's umbilical cord was taken aseptically from a healthy donor who had gone through a screening panel for HBsAg, HCV, Anti-HIV, TPHA, Anti-CMV IgG, Anti-Toxoplasma IgG, and clinical evaluation of disease factors. The baby's umbilical cord will be taken by cutting it about 10 cm long. The umbilical cord obtained was mixed into growth media containing 10% DMEM and HPL (human platelet lysate), then incubated at 37°C and 5% CO^2 for approximately 3 weeks. Every 3–4 days, the medium is replaced, until the cells reach a confluence of about 80%. The stem cells are then expanded until passage 5 and proceed towards quality control before use including identity, viability, purity, potency, and stability. Identity testing includes morphology, immunophenotyping, and differentiation ability of MSCs. MSCs have a spindle-shaped morphology, like fibroblasts, can express CD90, CD73, CD105, and Lin Negative, and have the ability to differentiate into adipocytes, chondrocytes, and osteocytes. Purity testing includes the detection of endotoxin and mycoplasma. Endotoxin and mycoplasma detection results must show negative results. Potency testing includes the ability of the SPM to produce paracrine effects. Stability testing includes karyotype examination. For viability, the minimum criteria are >80%.

For timing administration, the optimal timing of injection is best before scar formation in the ventricular wall. However, too early of

administration should be avoided as physiological mechanisms at that time are conducive for cells to migrate into an inflammatory microenvironment, which makes it unfavorable for cell survival and reduces its efficacy. A potential option to circumvent this issue of cell survivability and efficacy loss during early administration is by employing additional strategies that strengthen the stem cell during transplantation. Several available strategies include the pre-treatment of stem cells with serum deprivation (SD), hypoxia, some pharmacological agents, or even cryoprotective factors (Baldari et al., 2017). In a study done by Alijani-Ghazyani et al. (2020), overexpression of the cryoprotective factor Lipocalin 2 (Lcn2) is able to increase MSC survivability by acting as an antioxidant, anti-apoptotic, and anti-inflammation for the stem cells. Furthermore, the potential loss of homing mechanism of the stem cell during transplantation into STEMI is improved through Lcn2, as the factor is shown to increase stem cell adhesion capability and provide stress protection. Various other reported benefits of Lcn2 assisting in MSC transplantation in STEMI are mitigating LV remodeling, alleviating I/R-induced tissue damage, and reducing apoptotic cells due to oxidative stress. Although Lcn2 has shown promising novel outcomes, the proper mechanism of Lcn2 protective mechanism is still being researched and its effects are known mostly in non-clinical studies, though there are other experimental clinical studies on different diseases (Alagesan et al., 2022; Hermann et al., 2019; Roudkenar et al., 2018). The study by Yang et al. (2023) reported that the best time for MSCs transplantation was 1 week after infarction. Therefore, for the injection timing in this study, we chose between 6 and 7 days after STEMI.

C. Study Treatment and Follow-Up Results

In this study (number KET-245/UN2.F1/ETIK/PPM.00.02/2019), we reported four male patients with STEMI marked with symptoms, such as chest pain, cold sweat, and shortness of breath. The patient received either an intracoronary or intravenous umbilical cord-derived mesenchymal stem cells (UC-MSCs) injection. Administration via

intracoronary was administered by a stop-flow technique via an over-the-wire balloon catheter positioned within the stent portion while the intravenous route is injected directly into the vein using a syringe. All the written informed consent of patients was recorded, and this study was funded individually by the patient and with the assistance from PT. Prodia StemCell (ProSTEM), Jakarta, Indonesia.

1. Injection of UC-MSC

Case 1:

A 48-year-old male patient was admitted because of anterior STEMI. Left ventricular ejection fraction (LVEF) examined by MRI was 39%. One week after PCI, the patient was treated with around 50×10^6 UC-MSCs via the intracoronary route. There was no adverse event at two weeks post-transplantation.

Case 2:

A 45-year-old male patient was diagnosed with anterior STEMI and hypertension. Echocardiogram showed severe hypokinesis of the apical lateral and mid-septal wall with an LVEF of 45%. The LVEF was also 44% by MRI. After undergoing successful PCI, the patient was treated with intravenous UC-MSCs with a dose of 2×10^6/kg weight body (214×10^6) one week later. There was no adverse event at two weeks post-transplantation.

Case 3:

The patient was a 54-year-old male with acute anterior STEMI, hypertension, and diabetes mellitus. LVEF examined by MRI and echocardiogram was 20% and 24%, respectively. One week after PCI, the patient was treated with an intravenous infusion of 152×10^6 (2×10^6/kg weight body) of UC-MSCs. There was no adverse event that happened 2 weeks following the transplantation.

Case 4:

A 78-year-old male patient was diagnosed with anterior STEMI. On echocardiogram, the LV was grossly normal in size, and there was moderate to severe septal hypokinesis. The patient then underwent

Table 6.1 Summary of Case Illustration

Subject	Age	Gender	Diagnose	Intervention	Result			
					LVEF (%)		6MWT (m)	
					Before	After	Before	After
Case 1	48-year-old	Male	Anterior STEMI	Intracoronary	39	40	522	562
Case 2	45-year-old	Male	Anterior STEMI and hypertension	Intravenous	44	52	500	390
Case 3	54-year-old	Male	Acute anterior STEMI, hypertension, and diabetes mellitus	Intravenous	20	42	453	578
Case 4	78-year-old	Male	Anterior STEMI	Intracoronary	35	40	336	410

PCI and after the course of one week, was injected with 50×10^6 UC-MSCs via intracoronary route. The summary of case illustration is shown in Table 6.1.

2. Left Ventricular Ejection Fraction (LVEF)

The left ventricular ejection fraction (LVEF)–calculated as the stroke volume (end-diastolic volume minus end-systolic volume) divided by the end-diastolic volume–remains the main driver for categorizing heart failure (HF) and it is a cornerstone in all randomized clinical trials for patients with HF. Although LVEF has many acknowledged limitations, it remains key for the classification, stratification, management, and surveillance of HF during follow-up because it is easy to obtain and non-invasive. LVEF is a pivotal measure for managing HF by HF specialists and general cardiologists, but beyond cardiologists, it is well known and understood by a majority of internists, general practitioners, and geriatricians. Left ventricular ejection (LVEF) was examined before therapy, 6 months, and 12 months after therapy using MRI and echocardiogram. In CASE 1, LVEF examined by MRI was 39% and LVEF examined by echocardiogram was 38%, as a baseline. After six-month post-transplantation, an MRI examination showed a stable LVEF at 38%. At twelve months of observation by MRI, the LVEF remained unchanged at 37%. Echocardiogram before stem cells treatment showed septal and apical akinesis; with LVEF at 38%. Six

months of observation by echocardiogram showed an LVEF value of 40%. At 12 months, post-transplantation showed left ventricular dilatation and LVEF of 40%. In CASE 2, the echocardiogram showed severe hypokinesis of the apical lateral and mid-septal wall with LVEF of 45%. The LVEF was also 44% by MRI. Magnetic resonance imaging at 12 months demonstrated improvement of LVEF to 52%. Defects of the perfusion area and viability area were stable. Echocardiogram LVEF improved to 50% at 6 months. In CASE 3, LVEF examined by MRI and echocardiogram was 20% and 24%, respectively. The LVEF shown by MRI had increased from 20% to 37% in 6 months, and 42% in 12 months whereas the LVEF result shown by echocardiogram had increased from 24% to 37% in 6 months, and 45% in 12 months. In CASE 4, MRI showed LVEF improvement from 35% at baseline, 41% at 6 months, and 40% at 12 months after transplantation. At the same time, LVEF examined using an echocardiogram showed an improvement from 45% at baseline to 51% at 6 months, and 52% at 12 months (Figure 6.1).

Based on the result of our study, the overall LVEF was significantly higher in using the echocardiography method of measurement compared to that from MRI. Significant change of LVEF value through stem cell injection occurs mostly during the six months interval compared to the final 12 months, in which the value either increases or decreases significantly (MRI-6 months: +10.3; n=3; ECHO-6 months: +6.5; n=4), with CASE 1 being an exception for MRI, where the value consistently dropped across the 6–12-month intervals (MRI-6 months= -2). Prominent LVEF difference was also noticed between the measurement method, with MRI measuring lower LVEF compared to echocardiography. CASE 2 was an exception to these differences as the LVEF was higher in MRI compared to that of echocardiography (MRI-6 months: 52 > ECHO-6 months: 50).

In terms of scan accuracy, LVEF results from MRI scans seem to produce the most accurate results. In contrast, although echocardiographic measurements produce higher LVEF results, there is very low accuracy in its evaluation due to input results on

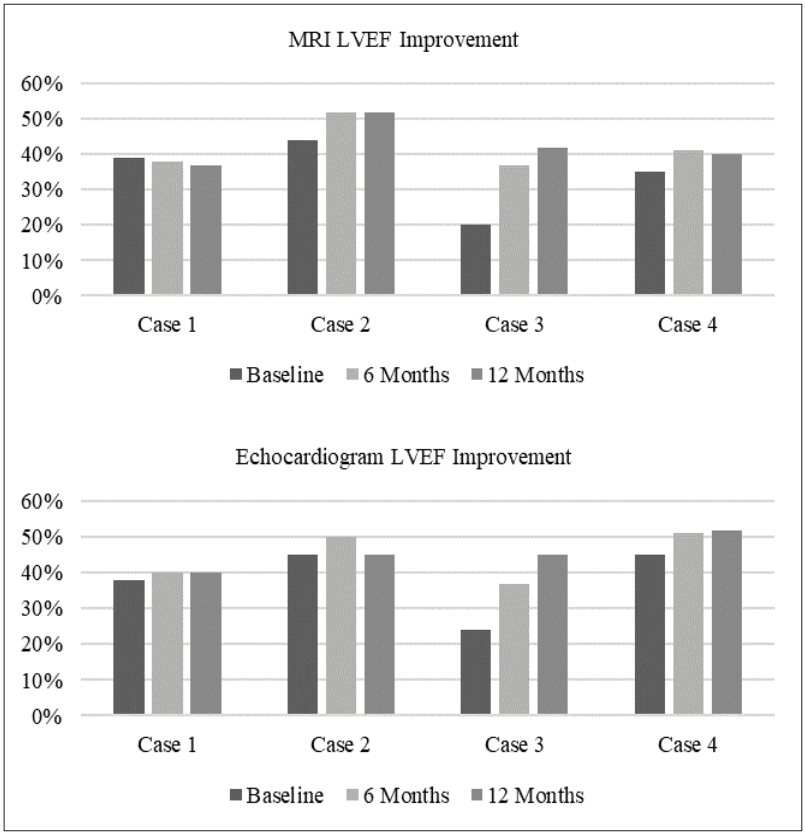


Figure 6.1 LVEF examined by MRI and echocardiogram.

the measurement having high heterogeneity and being subjective to each radiologist. Furthermore, echocardiography results have two cases identified as STQA (status quo ante) which has no significant or identifiable increase across the LVEF result measurement (CASE 1 and CASE 4), while MRI only has one case that is STQA (CASE 1), with the rest of the cases having a significant increase across the LVEF result. Additionally, using the heart failure guideline range (Heidenreich et al., 2022), categorical assessment of LVEF value is possible through the accurate result of MRI. From this, cases one to four was determined as reduce (n=<40; CASE 1: 39–37), mildly reduce

to preserve (n=40–50–n=>50; CASE 2: 44–52), and reduce to mildly reduce heart failure for both case three and four (n=<40–n=40–50; CASE 3: 20–42; CASE 4: 35–40).

Since there are limited participants in this case study, the discussion focus on stem cell changes, effects, and potentials towards myocardial infarction will be directed more towards individual case assessment, through various significant and interesting points within the MRI and ECHO scans data. Referring to MRI scans (Figure 6. 1), CASE 1 was shown to have no significant changes in allogeneic-umbilical cord mesenchymal stem cell (UC-MSC) treatment during the patient's LVEF across the 12 months of observation. According to the patient's MRI scans, the infarct size of the patient greatly exceeded the standard infarct size with a value that of >75%. This might suggest the decreased efficacy of the stem cell treatment leading to no significant LVEF changes during observation. Furthermore, the infarct size of other cases is only around the ≥50% range, hence the small increases in LVEF can be seen within the MRI scans data (Lee et al., 2020).

3. Quality of Life (Six-Minute Walking Test)

The six-minute walking test (6MWT) is a simple test that requires no specialized equipment or advanced training for physicians and assesses the submaximal level of functional capacity of an individual while walking on a flat, hard surface in a period of 6 min (6-minute walk distance; 6MWD). The 6MWT may be used as a tool for the measurement of a functional status of a patient especially in the case of advanced diseases with multiple comorbidities who cannot perform more complex exercise tests, such as patients with HF, chronic obstructive pulmonary disease, or cystic fibrosis. The prognostic role of 6MWT in terms of morbidity and mortality has been evaluated especially in patients with pulmonary arterial hypertension and in HF populations. The 6MWT should be performed preferably indoors, on a flat, straight, hard-surfaced corridor usually at least 30 m long. The 6MWT has been extensively used in various clinical studies in

the assessment of response to interventions in patients with HF as a measure to evaluate the effect of the treatment on a patient's functional status. It is considered to be an easy, widely available, and well-tolerated tool, yet with a questionable role in patients with HF, in contrast to populations of pulmonary arterial hypertension in whom 6MWT has been established as an important endpoint in clinical studies that led to therapy approval. The six-minute walking test was examined before therapy and at 2 weeks, 3 months, 6 months, and 12 months after therapy. In CASE 1, the 6-minute walking test results at baseline, 2 weeks, and 3, 6, to 12 months after injection were 522 m, 549 m, 561 m, 567 m, and 562 m respectively. In CASE 2, the 6-minute walking test results at baseline, 2 weeks, and 3, 6, to 12 months after injection, were 500 m, 518 m, 549 m, 593 m, and 390 m respectively. In CASE 3, the 6-minute walking test result had improved significantly from 453 m at baseline, 500 m at 2 weeks, to 639 m at 6 months. Unfortunately, it declined to 578 m at 12 months of observation. In CASE 4, the 6-minute walking test results at 2 weeks and 3, 6, to 12 months after injection were 336 m, 408 m, 324 m, and 410 m respectively (Figure 6.2). Overall, CASE 4 has the lowest recorded peak distance at 410m as opposed to other peaks of other cases (CASE 1: 567; CASE 2: 593; CASE 3: 639). One possible explanation for this decrease might be

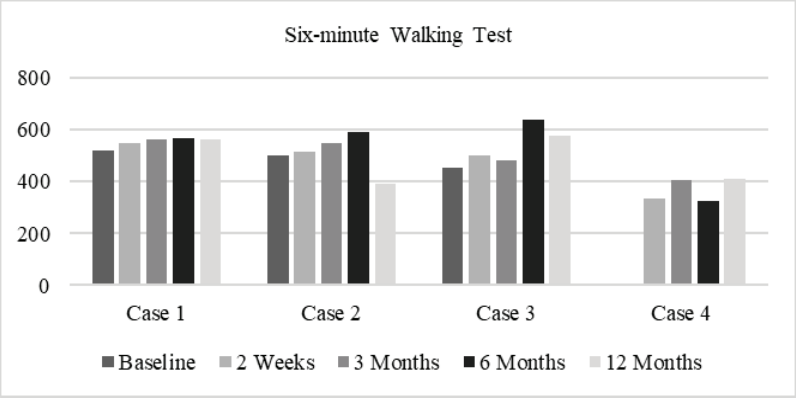


Figure 6.2 Quality of Life Assessment

that the subject in CASE 4 is significantly older than the subjects in the other cases (CASE 4, Age: 78), which generally makes it difficult to cover long distances due to lower stamina and physical conditions associated with old age.

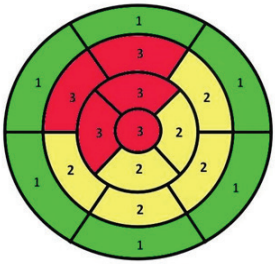
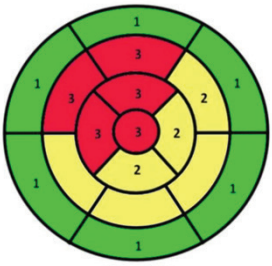
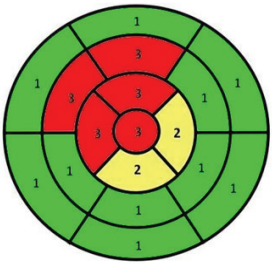
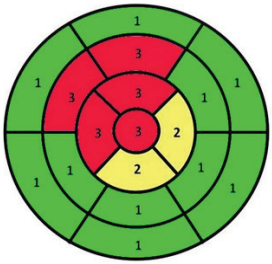
The result of the study is the same as the study by Bartolucci et al. (2017). Bartolucci's study used UC-MSC in patients with chronic stable heart failure with reduced ejection fraction (HFrEF) and showed that there was an improvement in left ventricular function, functional status, and quality of life in patients.

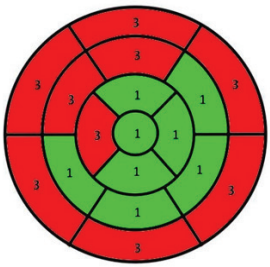
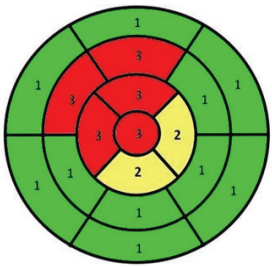
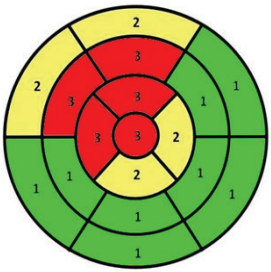
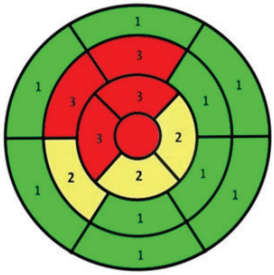
4. Regional Wall Motion Abnormalities

Additionally, the visualization of regional wall motion abnormality (RWMA) echocardiographic scans involves a quantitative assessment using scoring methods or an index, such as the wall motion score index (WMSI), which is calculated by dividing the sum of the score of each segment between the number of visualized segments (Gurunathan & Senior, 2017) and analyzed by two independent expert echocardiographers. In CASE 1, RWMA was consistent with previous anterior myocardial infarction, right ventricular contractility, and the valves were normal. No significant improvement of the WMSI value in the 12 months of observation seen in Table 6.2 can be found. Similarly, this can be said for CASE 2 and CASE 4, in which their WMSI value seen in the RWMA 17-segment model showed no improvement from the pre-treatment model scan towards the post-treatment scan (12 months; Table 6.2). Interestingly, only CASE 3 was shown to elicit significant improvement of its WMSI in the RWMA model, in which previous basal segments (anterior, anteroseptal, inferoseptal, inferior, inferolateral, anterolateral) that is labeled akinesis contractility improved into hypokinesis in the basal-anterior and anteroseptal segments, as well as into neurokinetic in the inferoseptal, inferior, inferolateral, and anterolateral segments, indicating a positive change through the stem cell treatment. While

the basal segments experienced improvement, the same cannot be said for the apical segments (anterior, septal, and lateral), in which the segments regressed into hypokinetic and akinesis contractility. The reasoning behind these changes is still being understood, however, the gradual loss of stem cell during transplantation on the injury site due to an inflamed environment is a potential cause for the gradual regression of interior apical segments, since the septal segments is further outside and reachable first by the stem cells allowing foremost repair (Alijani-Ghazyani et al., 2020; Kim et al., 2018).

Table 6.2 Regional Wall Motion Abnormalities (RWMA) and Wall Motion Score Index (WMSI) Result

Subject	Pre-treatment (Baseline)	Post-treatment (12 Months)
Case 1		
Case 2		

Subject	Pre-treatment (Baseline)	Post-treatment (12 Months)
Case 3		
Case 4		

D. The Role of Mesenchymal Stem Cells: Mechanism of Action

Studies suggest that MSC treatment benefits may be due to paracrine signaling. Paracrine signaling may play a role in promoting cardiomyocyte survival and increasing cardiomyocyte proliferation by inhibiting apoptosis. This statement is supported by a study in rats which proves that proteins such as VEGF, PDGF, IGF-1, and IL-1b secreted by MSCs can prevent cardiomyocyte apoptosis.

The role of MSCs in cardiac repair is also elicited through immunomodulatory and anti-inflammatory actions. When stem cells are injected into the myocardium, they suppress the expression of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, and monocyte chemoattractant protein-1, which reduces the inflammatory response to damage. The tissue inhibitor of metalloproteinase-1 and post-MI elevations in the expression of collagen-I and collagen-III have both

been demonstrated to be inhibited by MSC transplantation. The proliferation of cardiac fibroblasts and the generation of collagen-I and collagen-III from these cells are both severely inhibited by the paracrine compound secreted from MSCs. Thus, MSCs also play a role in preventing post-infarction remodeling.

Another important effect of MSCs for cardiac repair is inducing neovascularization. This process involves proteins, such as VEGF, FGF, and HGF (Bagno et al., 2018; Guo et al., 2020; Heinen et al., 2019; Hodgkinson et al., 2016).

E. Limitation of Study

The limitations of this study are the small number of participants and there are several factors identified through this study that can be attributed to the decrease and increase of the various assessments of STEMI patient functionality, such as LVEF value, WMSI scores, and the 6MWT. Infarct sizes as a factor influencing non-significant change in LVEF value (CASE 1) and old age affecting stem cell efficacy and homing properties, as well as reducing the distance traveled during 6MWT, is shown to be the noticeable factors affecting results in this study.

Furthermore, other factors affecting results in this study are speculated to be the injection route, in which both IV and IC injection methods differ in their results. As seen in Figure 6.1, two of the cases that experienced an increase in LVEF value are CASE 2 and CASE 3, while those that do not are CASE 1 and CASE 4. These two cases were given two different methods of stem cell injection, in which CASE 1 and CASE 4 experienced intracoronary injection (IC), while CASE 2 and CASE 3 experienced intravenous injection (IV). This result implies that cases that were given IC stem cell injection produced lower results, while those given IV injection experienced an increase. In many clinical studies, both IV and IC stem cell injections have been shown multiple times to yield significant and safe results in improving LVEF values, PET, or RWMA during in vivo studies on STEMI (Bartolucci et al., 2017; Gao et al., 2015; Peng et al., 2019).

Additionally, a systematic review by Lalu et al. (2018) of stem cell therapy on AMI confirms this same positive trend of both IV and IC injection methods being safe, effective, and viable for use.

At their core, IC and IV differ only in their injection route, which is through the direct path to the bloodstream for intravenous infusion and intracoronary administration through the direct path of the coronary veins (Lalu et al., 2018). Furthermore, both injection methods also seem not to produce any significant difference in their method of injection, aside from the fact that IV stem cells can get trapped during development in lung airways, such cause did not occur in this experiment, as our IV patients produced the significant increase (Liesveld et al., 2020; Schlundt et al., 2015). Although this is not significant to cause a large change in LVEF value, potential reasons that can be accounted for are additional statuses found in the intracoronary injection cases (CASE 1 and CASE 4). CASE 1 is affected with a larger infarct size that of >75%, requiring perhaps more stem cell dosage, and CASE 4 has additional affecting factors such as old age, which can attribute to influencing UC-MSC homing property, to which the stem cell injected might home in onto other organs that requires stem cell, and not towards the myocardial infarction site alone, thus requiring more stem cell dosage. Furthermore, this occurrence is likely to occur, as MSC homing efficiency is reported to be poor (<10%) in many various imaging studies, as well as previously said, that stem cells have the potential in getting trapped in lung pathways (Krueger et al., 2018; Liesveld et al., 2020). Another interesting instance in CASE 4 condition is that the patient was also diagnosed with a multi-vessel type blood vessel, as opposed to other cases that are only diagnosed with a single vessel, which again might suggest that the stem cell requirements for the treatment could potentially be higher than what is used. This factor could also be assisted in explaining the ineffective homing mechanism of MSCs coupled with old age, which makes the stem cell open to home into more areas that are needed.

Another potential factor is through the blood vessel type disease, which is a multi-vessel or single vessel, in which the multi-vessel type

was present in CASE 4, where most of the LVEF and 6MWT data was lower than other cases, potentially contributing as an affecting factor. As for case series with an improvement (CASE 2 and CASE 3), both cases have lower infarct size compared to CASE 1 with around ≥50% infarct size and are considerably younger in contrast with CASE 4, as well as having single vessel type. Therefore, implying a correlation between age, infarct size, and blood vessel type as factors affecting STEMI treatment using UC-MSC.

This limitation of the study can be improved in the future by choosing the best route of administration, doses, and timing of transplantation. The retention of stem cells also can be achieved by exercise training (ET). A study by Souza Vieira et al. (2020) showed that ET improves myocardial microenvironment for stem cell transplantation.

The findings of this study suggest that UC-MSC therapy has the potential to significantly improve clinical outcomes in AMI patients. This could lead to reduced mortality rates, fewer complications, and a better overall prognosis for individuals suffering from heart attacks. The observed reduction in myocardial damage following UC-MSC treatment highlights the possibility of preserving cardiac function and limiting post-AMI heart failure. This implies that UC-MSC therapy may represent a valuable addition to the standard treatment protocol for AMI. Moreover, the sustained improvement in left ventricular function seen in our study could translate into long-term benefits for AMI patients. This implies that UC-MSC therapy might contribute to enhanced cardiac rehabilitation and a better quality of life for survivors. Furthermore, our research underscores the importance of patient-specific approaches in AMI therapy. The identification of patient characteristics associated with a positive response to UC-MSC treatment suggests the potential for personalized medicine, where individuals can be stratified for the most effective treatment strategies. Combining UC-MSC therapy with other innovative treatments, such as gene therapy or cardiac rehabilitation programs, may further enhance outcomes. These findings imply that AMI treatment protocols

may evolve to incorporate a multi-modal approach for optimal results. Additionally, the observed safety and low incidence of adverse effects in UC-MSC therapy indicate that this treatment modality holds promise for broad application in clinical settings. Further studies should explore the long-term safety of UC-MSCs and determine the ideal dose and delivery method. Our results highlight the need for additional research to elucidate the precise mechanisms by which UC-MSCs confer their benefits in AMI. Future investigations can focus on optimizing cell dosing, timing of administration, and long-term outcomes to refine the clinical application of UC-MSC therapy.

F. Conclusion

The case series has successfully identified factors that may influence the outcomes of allogeneic umbilical cord mesenchymal stem cell (UC-MSC) treatment for STEMI. However, further research is needed to draw more definitive conclusions. The study's limitations include a small sample size of only four patients, making it challenging to generalize the results, despite the observed significant improvements. Additionally, incomplete radiological data and variations in results across different medical institutions introduced heterogeneity to the study, complicating the interpretation of similar results. To obtain more robust and compelling results, future research should involve longer follow-up periods and larger patient cohorts, specifically focusing on younger or early-stage STEMI patients. In conclusion, our study demonstrates that using allogeneic UC-MSCs as an adjunct treatment for anterior STEMI is safe and well-tolerated, offering promise for STEMI patients. However, additional research is essential to build upon these findings and address the identified limitations.

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