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Effects of Timing on the Efficacy of Stem Cell Transplantation after Acute Myocardial Infarction

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ABSTRACT

Acute myocardial infarction (AMI) is the blocking of coronary arteries that prevents oxygenated blood from reaching the heart tissues, resulting in damage to the myocardium and affecting heart function. This condition affects millions of people every year and is detrimental to their quality of life. Several clinical trials have investigated the efficacy of using bone marrow-derived stem cells (BMSCs) to improve heart function after AMIs. However, different variables could impact the results of the trials, one of them being the injection time of cell therapy after reperfusion. This paper aims to investigate the short-term effects of timing on the efficacy of bone marrow-derived mononuclear cell transplantation after acute myocardial infarction. A systematic literature search of PUBMED, EMBASE, European Society of Cardiology, and American Heart Association databases was made on randomized controlled trials with at least 3-month follow-up data for patients with AMI undergoing percutaneous coronary intervention (PCI) and receiving intracoronary autologous BMSC transfer subsequently. A total of 12 trials with 1061 patients were selected for analysis. Compared to baseline level, BMSC transfer within 24 hours of PCI significantly improved left ventricular ejection fraction (LVEF; 3.44% increase, 95% confidence interval [CI]: 2.20%-4.68%, P< 0.00001). The "3-7 days after PCI" subgroup also showed notable improvements in LVEF (LVEF; 2.52% increase, 95% confidence interval [CI]: 1.01%-4.04%, P = 0.001). However, in the subgroups that received BMSC transplantation either 7-14 days after PCI or later than 15 days after PCI, there was no significant effect on treatment outcome.

Background

According to the World Health Organization, cardiovascular disorders are the leading cause of death globally, taking an estimated 17.9 million lives each year. This number has significantly increased after the COVID-19 pandemic, which has brought great relevance to the topic. Over the past decades, numerous studies have suggested that after an acute myocardial infarction (AMI), the infusion of bone marrow-derived mononuclear cells (BM-MNCs) could improve heart function by promoting tissue repair. There has been a debate over the true efficacy of BM-MNCs therapy, with some trials like REPAIR-AMI (Schächinger, et al., 2006) and TOPCARE-AMI (Assmus, et al., 2002) demonstrating the feasibility of this treatment and obtaining positive results, while other trials like the Late TIME (Traverse, et al., 2011) and the BONAMI trial (Roncalli, et al., 2010) failing to show myocardial improvement after the follow-up analysis.

This discrepancy in results could be related to the lack of standardization of variables between the trials. One example of these variables is the injection time of the BM-MNCs after reperfusion, which varies from study to study as the optimum injection time to enhance myocardial function has not been established. The injection time of the cell therapy could have a great impact on the efficacy of the treatment, and establishing an ideal period of time to deliver the BM-MNCs may help save and improve the lives of millions of people who suffered from this condition. Therefore, the aim of this research is to determine the optimum time to deliver the BM-MNCs after an acute myocardial infarction, while investigating the possible biological factors responsible for this phenomenon.

Acute Myocardial Infarction (AMI)

The myocardium is a muscle layer responsible for the pumping contractions of the heart. It is composed of cardiac muscle cells (called cardiomyocytes), extracellular matrix, capillary microcirculation and fibroblasts, which are responsible for forming connective tissue (Sutton and Sharpe, 2000). Cardiac cells, just like every other cell in the human body, need energy in the form of adenosine triphosphate (ATP) to perform their biological functions. In this process, called cellular respiration, oxygen molecules have a vital role in breaking down glucose, as oxygen is the final electron acceptor that receives the transferred electrons, which is needed to complete the reaction. Therefore, the heart muscle relies on a steady flow of oxygen-rich blood to maintain its normal pumping rate.

A myocardial infarction, commonly known as a heart attack, is a condition in which the blood flow in the heart muscle is severely reduced or cut off completely. This happens when a plaque, a fatty substance made up of deposits of cholesterol and other substances, starts building up on the walls of coronary arteries. When the plaque ruptures, a blood clot is formed, blocking the artery. The blood supply to the heart is cut off and muscle tissue starts to die due to the lack of oxygen. The damage or death of part of the heart muscle caused by the lack of blood flow is called myocardial infarction (Represented in Figure 1).

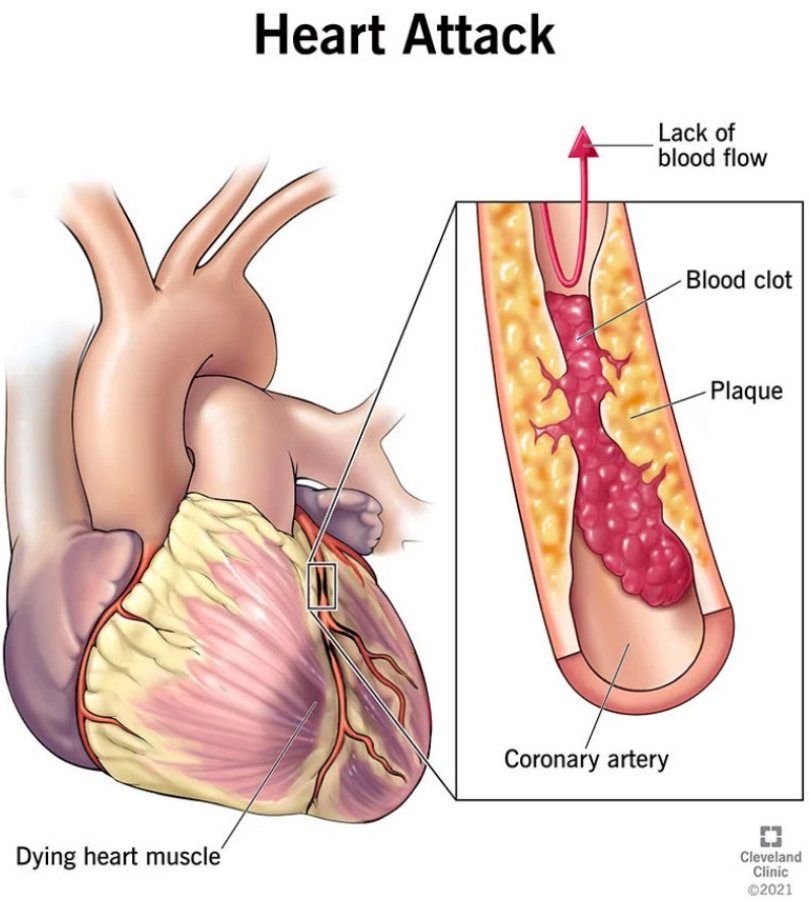

Figure 1. Visual representation of a myocardial infarction (heart attack).
Image source: https://my.clevelandclinic.org/health/diseases/16818-heart-attack-myocardial-infarction

The heart is the organ responsible for pumping blood around the body in order to supply oxygen and nutrients to the cells and remove metabolic waste. As a consequence of myocardial infarctions, cardiac muscle cells go under

hypoxic conditions and are unable to produce sufficient ATP. The result is that their contractions become irregular and uncoordinated; they do not pump blood effectively. The reduced blood flow to the organs can be associated with several health problems, as well as resulting in shortness of breath, fatigue and swelling. (Peteiro et al., 2011).

The human heart has a very limited natural capacity to regenerate the tissue that has been lost by injury (Renuka and Sethu, 2015). Instead, in the weeks following the AMI, a large, collagen-rich scar tissue forms in the place of the damaged tissue. However, cardiac scarring can interfere with the heart's contraction rate, increasing the probability of future heart related issues like aneurysms, additional MI events and organ failure. (Kikuchi and Poss, 2012).

Bone Marrow Mononuclear Cells (BM-MNCs)

Current studies have shown that stem cell therapy is associated with the improvement of heart function following a myocardial infarction. Bone marrow mononuclear cells (BM-MNCs) are a mixed population of single nucleus cells, including stem cells such as hematopoietic, mesenchymal and progenitor cells (Kikuchi-Taura, et al., 2020). Stem cells have two unique characteristics that favor their use in regenerative medicine: they have great capacity for self-renewal and can differentiate into various cell types. In this case, the stem cells found in bone marrow are capable of differentiating into cells like myocytes and fibroblasts, which are cells that are needed for heart muscle repair (Caplan and Dennis, 2006).

Initially, the BM-MNCs were thought to restore the damage in infarcted hearts only by differentiating themselves into cardiomyocytes, to replace the ones that had been damaged. However, recent studies have identified that the importance of stem cells in AMIs goes beyond differentiating into new cardiomyocytes, as they secrete a variety of proteins that enhance regenerative processes through paracrine interactions (Mirotsou, et al., 2011).

Paracrine Interactions

Paracrine factors are diffusible proteins, such as cytokines or growth factors, capable of producing signals that can induce changes in nearby cells. When those proteins diffuse into neighboring cells and modify them, this event is called a paracrine interaction (Figure 2) (Gilbert, 2015). This interaction is very important in myocardium repair because it allows cells to communicate and influence each other, creating a unique microenvironment within the cardiac tissue (Hodgkinson, et al., 2016). It is likely that paracrine factors are released in a spatial and time-related way to develop different biological effects in order to meet the environmental needs of the tissue after an injury (Gnecchi, et al., 2008).

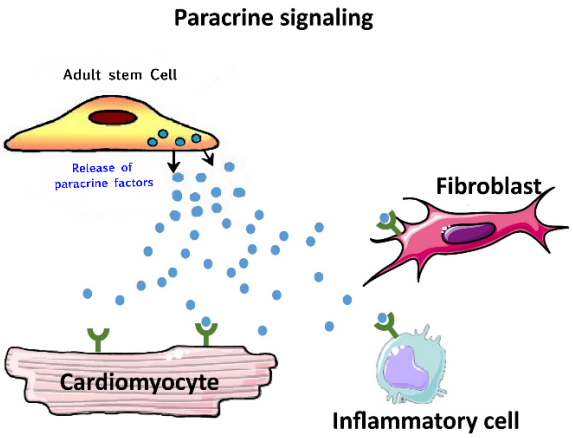

Figure 2. Representation of the process of paracrine interactions. Image adapted from: Segers & De Keulenaer. (2021) and Gnecchi et al. (2008)

Thus, paracrine interactions improve cell communication and promote a series of complex reactions, which can lead to tissue repairing and remodeling, apoptosis and fibrosis inhibition and cardioprotective processes, as well as promoting the formation of new blood vessels (Figure 3) (Sid-Otmane, et al., 2020). Consequently, paracrine factors participate in many activities that are vital to myocardial tissue regeneration.

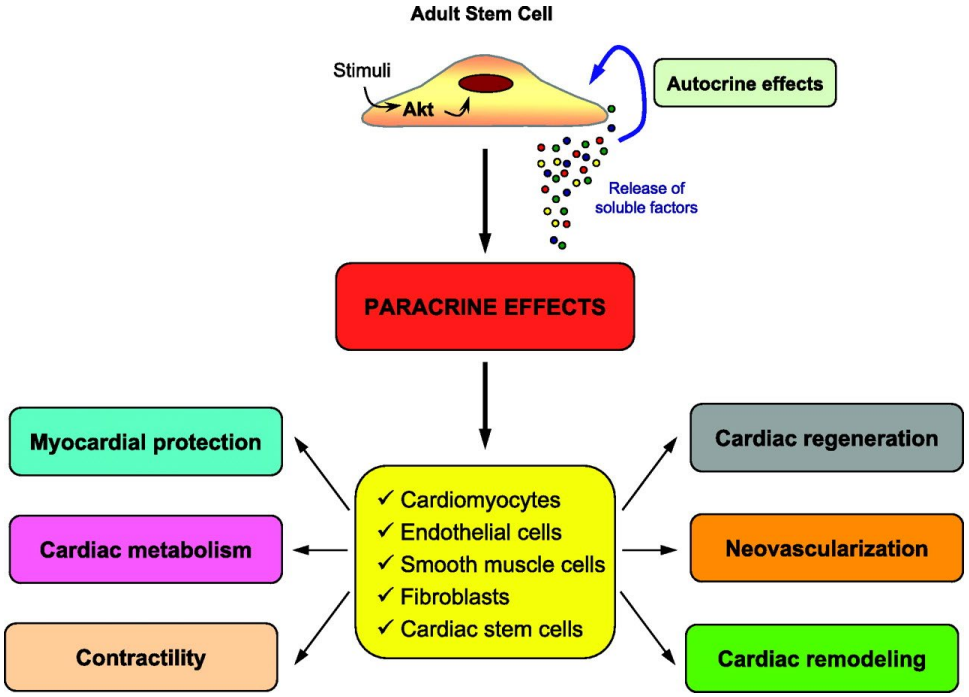

Figure 3. Flowgram representing the different cells that respond to paracrine factors and the outcome of the interaction. Image source: Gnecchi et al. (2008).

Therefore, there is biological evidence that supports the feasibility and efficacy of BM-MNC therapy after AMIs. Despite that, there are many factors that could interfere with the success of the treatment, such as the stem cell type, the number of cells injected, the overall condition of the patients and the method and timing of the transplantation. Although many researchers over the decades have been studying this topic, the ideal conditions needed to achieve the best results with this treatment have yet to be established. This investigation was made in order to further analyze the effects of timing on the efficacy of the treatment and the possible biological explanations for any effects observed.

Methodology

The left ventricular ejection fraction (LVEF) was chosen as the primary indicator of this study, since it allows an overall quantitative measurement of heart function. LVEF is an important biological indicator because it determines the heart's ability to distribute oxygen-rich blood to the rest of the body by measuring the amount of blood that is pumped out of the left ventricle each time the heart contracts. After a myocardial infarction, the damaged cardiac cells affect the heart's ability to pump blood, therefore lowering LVEF levels. BM-MNCs therapy is suggested to promote myocardial repair, which would contribute to the enhancement of heart function, consequently, increasing LVEF levels. Thus, LVEF improvement from baseline to follow-up will be used to determine the success of each BM-MNCs delivery time by providing a quantitative analysis of myocardial repair in each subgroup.

Additionally, a number of different variables could impact the outcomes of the trials, so aiming to further specify the scope of this investigation, only the trials in which the injected cells were autologous, and the delivery route was intracoronary were considered for the analysis. Autologous cells are taken from the patient's own bone

marrow, thus they offer a lower risk of complications after the procedure, such as infections or the patient's body rejecting the infused cells (Jansen of Lorkeers, et al., 2015). The intracoronary injection is administered within the coronary arteries of the heart and is suggested to be the most efficient method of cell therapy delivery due to the direct site of injection, which would allow a higher number of transferred cells to reach the target tissue (Perin, 2016).

Search Strategy

In order to retrieve the most relevant studies on randomized controlled trials of intracoronary autologous BM-MNCs used in the treatment of acute myocardial infarction, a complex search was done using the database from PUBMED, EMBASE, European Society of Cardiology (ESC) and American Heart Association. The keywords used to retrieve the literature were “stem cell therapy, bone marrow mononuclear cells, acute myocardial infarction, autologous cells, intracoronary infusion”. All relevant studies published in English, Portuguese or Spanish were included.

Selection Criteria

Studies were included if they met the following criteria: (1) type of study: clinical trials with randomized controlled design and at least a 3-month, 4-month or 6-month follow-up data after baseline. (2) object of research: patients admitted within 24 hours after the onset of clinically diagnosed acute myocardial infarction. The experimental group received BM-MNCs transplantation, regardless of the dose administered. The cases in the control group received standard medical treatment without BM-MNCs transfer. (3) AMI primary treatment: Both the experimental and the control groups underwent percutaneous coronary intervention (PCI). (4) outcome indicators: Left ventricular ejection fraction (LVEF) measured by cardiac magnetic resonance imaging (CMRI), echocardiogram (ECHO), single-photon emission computed tomography (SPECT) or left ventricular angiography.

The trials were excluded if: (1) cell transplantation was through intravenous or intramyocardial injection. (2) injection of mesenchymal stem cells (MSCs) or progenitor cells mobilized from bone marrow.

Data Extraction and Statistical Analysis

The following characteristics were extracted from each clinical trial: study design, baseline participants characteristics, intervention strategies and left ventricular ejection fraction (LVEF) levels.

Intergroup analysis was based on the difference of average LVEF from baseline to follow-up between the BM-MNCs and control groups. In order to investigate the impact of the BM-MNCs injection time, a subgroup analysis was performed based on the timing of transplantation after percutaneous coronary intervention (PCI). PCI is a standard medical procedure for reperfusion, which is the restoration of blood flow after a period of ischemia or lack of oxygen. The LVEF change in all the trials was compared using the Review Manager (RevMan) 5.4 software. The subgroups were divided as “within 24 hours of PCI”, “between 3 and 7 days after PCI”, “between 7 and 14 days after PCI” and “over 15 days after PCI”.

Results

Quantitative Results

Using the search strategies, 1,306 publications were identified, and 10 articles were included in this investigation, out of which 12 randomized controlled trials were selected for analysis. The total number of patients was 1061 of whom

565 received BM-MNCs treatment. The primary indicator of this analysis will be the LVEF, and since there are several different methods of measuring its levels, the method used in each trial is identified in Table 1.

Table 1. Table containing the baseline characteristics of the studies included in the analysis.

Clinical Trial	Sample Size (BMC/Control)	Mean age (years)	Male (%)	Cell type	Number of cells	Cells injection time	Follow-up (months)	LVEF measuring tool	Average Baseline LVEF
Assmus, et al. 2002 TOPCARE-AMI	(9/11)	55	85.5	BM-MNC	7.35×10^6	4.3 days after PCI	4	LV angiography	51.3%
Choudry, et al. 2015 (REGENERATE-AMI)	(55/45)	56.6	85	BM-MNC	5.98×10^7	Within 24 hours of PCI	3	CMRI and Computed Tomography	48.54%
Grajek, et al. 2009	(31/14)	50.4	84	BM-MNC	4.10×10^8	4-6 days after PCI	3	ECHO and SPECT	50.6%
Huang, et al. 2006	(27/25)	59.4	63	BM-MNC	1.80×10^8	Within 24 hours of PCI	6	ECHO and SPECT	44.7%
Janssens, et al. 2006	(33/34)	56.9	82	BM-MNC	3.04×10^8	Within 24 hours of PCI	4	CMRI	47.7%
Roncalli, et al. 2011 (BONAMI)	(52/49)	55.5	85.3	BM-MNC	9.83×10^7	7-10 days (9.3 days) after PCI	3	SPECT and Radionuclide Angiography	39%
Schächinger, et al. 2006 (REPAIR-AMI)	(101/103)	56	82	BM-MNC	2.36×10^8	3-7 days (4.35 days) after PCI	4	LV angiography	47.1%
Sürder, et al. 2013 (early SWISS-AMI)	(65/67)	55.5	84.9	BM-MNC	1.60×10^8	5-7 days After PCI	4	CMRI	37.1%
Sürder, et al. 2013 (late SWISS-AMI)	(63/67)	59	83.1	BM-MNC	1.40×10^8	3-4 weeks after PCI	4	CMRI	37.6%
Traverse, et al. 2011 (Late TIME)	(58/29)	56.1	84	BM-MNC	1.47×10^8	2-3 weeks (17.1 days) after PCI	6	CMRI	47%
Wollert, et al. 2017 (Low-Dose)	(38/26)	54	89.5	BMCs (including BM-MNC)	7.00×10^8	8.1 days after PCI	6	CMRI	45%
Wollert, et al. 2017 (High-Dose)	(33/26)	56	88.5	BMCs (including BM-MNC)	20.6×10^8	8.1 days after PCI	6	CMRI	45%

For each trial, the results of the difference in average LVEF change and its standard deviation (from baseline to follow-up) within the group of patients who received stem cell treatment and within the group that received control treatment were calculated and expressed in Table 2.

The standard deviation values of the change in LVEF from the Assmus, et al. and Grajek, et al. trials were not available in their respective articles. Thus, these values were estimated using a mathematical range calculated based on the standard deviation of the initial LVEFs (baseline) and final LVEFs (follow-up) as follows:

If, $\sigma^2 initial = \frac{\sum (x_i - \overline{x})}{n}$ and $\sigma^2 final = \frac{\sum (y_i - \overline{y})}{n}$, then

$$\sigma_{\triangle}^2 = \frac{1}{n}\sum (y_i - \overline{y})^2 + \frac{1}{n}\sum (x_i - \overline{x})^2 - \frac{2}{n}(y_i - \overline{y})(x_i - \overline{x})$$

Using Cauchy–Schwarz's Inequality, we have that,

$$\sigma_{\triangle}^2 = -2\sqrt{\frac{\sum (y_i - \overline{y})}{n}}\sqrt{\frac{\sum (x_i - \overline{x})}{n}} \leq (y_i - \overline{y}) \times (x_i - \overline{x}) \leq 2\sqrt{\frac{\sum (y_i - \overline{y})}{n}}\sqrt{\frac{\sum (x_i - \overline{x})}{n}}$$

$$|\sigma final - \sigma initial| \leq \sigma_{\triangle} \leq \sigma final + \sigma initial$$

The average value from this range was used as the estimated standard deviation for the change in LVEF in the Assmus, et al and Grajek, et al trials.

Analyzing the graph and the data shown on Figure 4, it is possible to observe that LVEF levels had an overall increase of 1.64% (95% confidence interval [CI]: 0.51%-2.77%, P = 0.004), which demonstrates the general efficacy of the treatment. The subgroup that showed the biggest LVEF improvement from baseline to follow-up was the BM-MNCs injection within 24 hours of PCI, with a significant change of 3.44% (95% CI 2.20%-4.68%; $p < 0.00001$). The "3-7 days after PCI" subgroup also showed notable improvements in LVEF, with a 2.52% increase (95% CI 1.01%-4.04%, P = 0.001). In contrast, the "over 15 days after PCI" injection subgroup showed the lowest improvement, resulting in a negative outcome of -0.71% (95% CI -4.90%-3.48%; $p = 0.74$), that indicates a bigger enhancement in the control group compared with the BM-MNCs group. The "7-14 days after PCI" showed no significant effect on LVEF improvement, with a slight increase of 0.61% (95% CI –0.28%-1.50%; $p = 0.18$). Additionally, it is possible to observe a trend in the subgroups, in which the LVEF improvement decreases as the time taken for the injection increases, with the "within 24 hours of PCI" subgroup obtaining the best results, followed by the "3-7 days after PCI" subgroup, then the "7-14 days after PCI" subgroup and, lastly, the "over 15 days after PCI" subgroup.

Table 2. Comparison between baseline and follow-up LVEF levels within the BM-MNCs group and the Control group. The changes in LVEF levels were expressed with adjustments based on an ANCOVA model, following the data format obtained from the clinical trials.

* Estimated values

	BM-MNCs Group				Control Group			
Clinical Trials	Average LVEF at Baseline (SD)	Average LVEF at Follow-up (SD)	Change in LVEF	Standard Deviation	Average LVEF at Baseline (SD)	Average LVEF at Follow-up (SD)	Change in LVEF	Standard Deviation
Assmus, et al. 2002 TOPCARE-AMI	51.6% (±9.6)	60.1% (±8.6)	8.5%	8.6*	51% (±10)	53.5% (±7.9)	2.5%	7.9*
Choudry, et al. 2015 (REGENER-ATE-AMI)	47.5% (±2.6)	53.3% (±2.9)	5.7%	3.1	49.2% (±3.1)	49.9% (±3)	1.6%	3.3
Grajek, et al. 2009	50.32% (±9.8)	50.03% (±8)	-0.3%	8*	50.84% (±12)	48.91% (±11)	-1.93%	11*
Huang, et al. 2006	44.7% (±3.9)	50.4% (±4.7)	5.6%	3.3	43.5% (±3.5)	45.9% (±5.4)	2.4%	3.2
Janssens, et al. 2006	48.5% (±7.2)	51.8% (±8.8)	3.4%	6.9	46.9% (±8.2)	49.1% (±10.7)	2.2%	7.3
Roncalli, et al. 2011 (BONAMI)	35.6% (±7.0)	38.9% (±10.3)	3.3%	8.8	37.0% (±6.7)	41.3% (±9.0)	4.3%	7.9
Schächinger, et al. 2006 (REPAIR-AMI)	48.3% (±9.2)	53.8% (±10.2)	5.5%	7.3	46.9% (±10.4)	49.9% (±13)	3%	6.5
Sürder, et al. 2013 (early SWISS-AMI)	36.5% (±9.9)	37.9% (±10.3)	1.8%	8.4	40.0% (±9.9)	39.6% (±12)	-0.4%	8.8
Sürder, et al. 2013 (late SWISS-AMI)	36.3% (±8.2)	37.4% (±9.7)	0.8%	7.6	40.0% (±9.9)	39.6% (±12)	-0.4%	8.8
Traverse, et al. 2011 (Late TIME)	48.7% (±12)	49.2% (±13)	0.5%	8.2	45.3% (±9.9)	48.8% (±7.8)	3.6%	9.3
Wollert, et al. 2017 (LD)	44.2% (±7.8)	48.2% (±9.7)	3.8%	2.25	47.8% (±6.7)	50.4% (±7.0)	3.3 %	2.75
Wollert, et al. 2017 (HD)	44.8% (±9.1)	49.2% (±10.6)	4.3%	2.4	47.8% (±6.7)	50.4% (±7.0)	3.3 %	2.75

The data presented in Table 2 was used on the RevMan software to create the statistical analysis and the graph expressed in Figure 4.

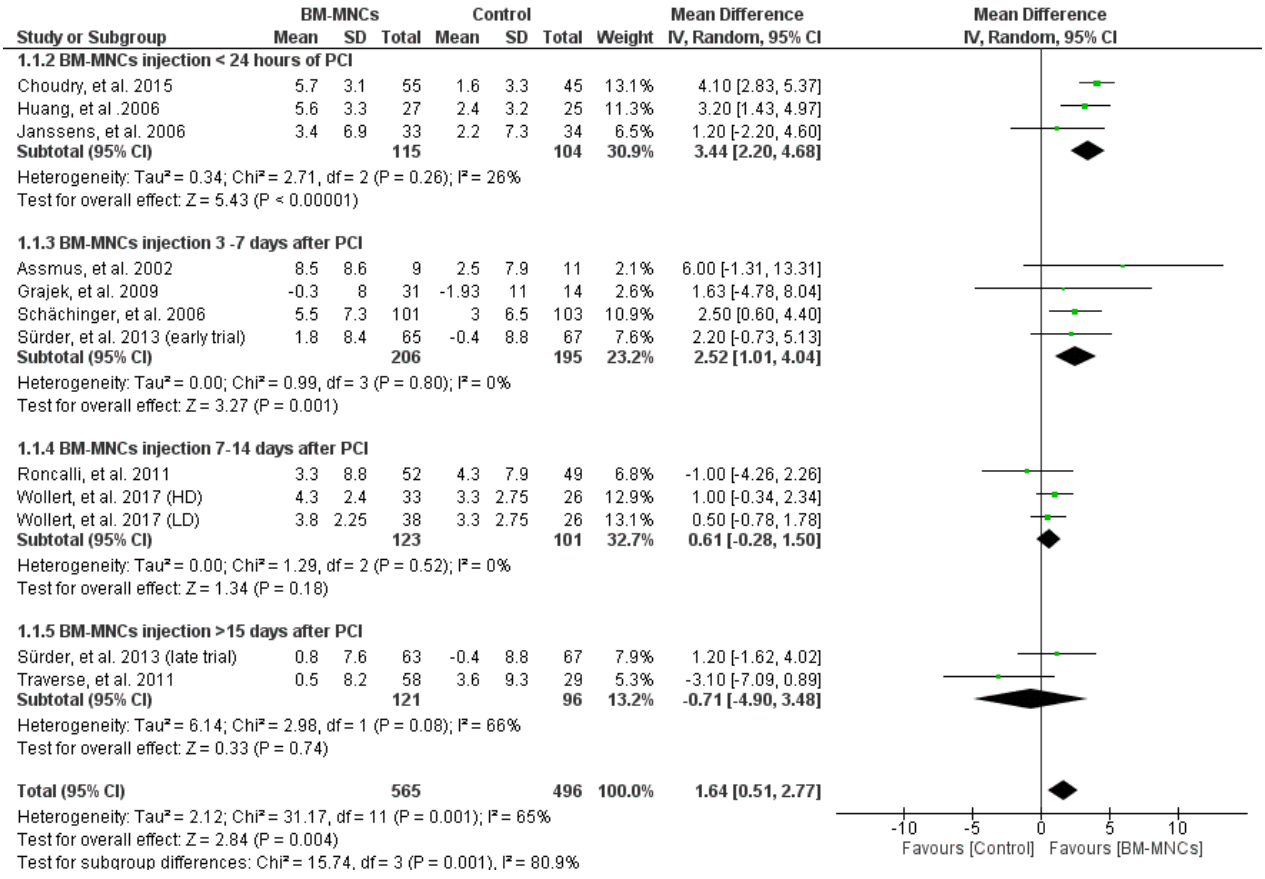

Figure 4. Forest plot comparing the impact of the different time delivery groups on the LVEF improvement from baseline to follow-up.

Biological Analysis

After myocardial ischemia, an intense inflammatory response is triggered by the tissue damage and is intensified by the process of PCI. This inflammatory reaction is observed within hours of reperfusion and declines by day 5 (Frangogiannis, 2002).

Inflammation happens when the damaged cells release chemicals that cause blood vessel dilation, higher blood flow and an increased capillary permeability, which allows fluids and proteins to seep through the vessels and reach the injury site. The inflammatory reaction then triggers the activity of paracrine factors (that were previously discussed) to help mediate the inflammation (Seropian, et al., 2014). These signaling proteins, such as cytokines and growth factors, are produced by several different cells, including bone marrow mononuclear cells. The production of these proteins is also increased after overcoming hypoxic conditions like myocardial infarctions, in which the levels of oxygen in the tissue are very low (Kinnaird, et al., 2004).

It has been shown that cytokines and growth factors secreted by the BM-MNCs help in the processes of differentiation, proliferation, wound healing and angiogenesis (Kusuma, et al., 2017). The differentiation and proliferation factors can improve heart function by accelerating the process of replacing the damaged or necrosed cardiomyocytes. Additionally, the development of new blood vessels can help maintain the homeostatic balance of the heart, as angiogenesis plays an important role in protecting the surviving cardiomyocytes from post-ischemic apoptosis (Tang, et al., 2004).

Therefore, as the inflammation begins within hours of reperfusion and lasts until day 5, the triggered inflammatory process during this period would intensify the production and secretion of paracrine factors, which would then act on the heart muscle by repairing the damaged tissue.

Thus, these processes would further explain the results obtained from the meta-analysis. As paracrine factors are produced by the BM-MNCs, the injection of these cells within 24 hours of reperfusion, when the inflammatory reaction is intensive, would increase the production of cytokines and growth factors in the damaged tissue due to the strong stimulus coming from the inflammation. These proteins will promote paracrine interactions, which will then act on the tissue repair. As a consequence, the higher number of available cytokines and growth factors cells would result in a more efficient regeneration of the myocardium.

Accordingly, the results of the clinical trials analysis show that the longer it takes for the BM-MNCs to be injected, the more LVEF enhancement decreases. This aspect can be observed as the subgroup that obtained the highest LVEF improvement was the one with the shortest injection time after PCI, and the subgroup that obtained the lowest LVEF improvement was the one with the longest injection time after PCI. Thus, this investigation allows us to suppose that the effects of BM-MNCs injection time can be explained by the inflammatory levels of the tissue, which presents high inflammation during the first days after the AMI and decreases as the days pass.

Discussion

This study shows that the injection of BM-MNCs within 24 of PCI have the best results in improving heart function after AMI. Through a biological investigation, it can be supposed that a probable reason for this is due to the biological process of inflammation that can stimulate the secretion of paracrine factors, which help regenerate the myocardium. The data shows that timing is a crucial factor, as once the inflammatory process is at its peak, the activity of cytokines and growth factors is intensified.

A number of different factors play a significant role in myocardium repair. These factors include the type of cells transplanted, the number of BM-MNCs injected, whether the BM-MNCs were autologous or allogeneic, the route of administration of the cells, the date of follow-up measurements and other external factors such as the patient's lifestyle and the presence of other medical conditions.

These factors can have big implications on the results of the trials, for example, the number of BM-MNCs injected can impact the number of paracrine factors in the injury, because if there are more cells available to produce them, then their production will be increased. Also, another factor that can influence the overall results is the date of the follow-up measurements, since the heart takes time to repair the tissue, if the follow-up dates are too divergent, then the trials will be compared at different developmental stages, disrupting the accuracy of the research. Additionally, the different lifestyles and other medical conditions between the patients can also affect the outcomes because external aspects such as diet or physical activity can influence the recovery of the myocardium (Krantz, 1980).

Conclusion

In conclusion, the aim of this research was to determine if the timing of BM-MNCs injections affected the efficacy of their use in AMI treatment and to investigate the possible biological reasons for any observed effects. For the purpose of this exploration, the myocardium repair after AMI was determined through heart function improvement, that was measured by LVEF levels.

Throughout the investigation, it is shown that the timing of BM-MNC injection is a vital element in the success of myocardium repair, as the meta-analysis of the trials revealed a correlation between the LVEF improvement, and the time taken to inject the mononuclear cells after PCI. The overall results expressed the efficacy and feasibility of this cell therapy in the treatment of AMI with the total LVEF average improvement of 1.64% (95% confidence interval [CI]: 0.51%-2.77%, P = 0.004).

The subgroup analysis was used to compare how different injection times affected LVEF enhancement. The highest average LVEF improvement was observed in the "within 24 hours of PCI" subgroup, with mean difference from baseline to follow-up of 3.44% (95% CI 2.20-4.68; $p < 0.00001$). In contrast, the subgroup that obtained the worst results was the "over 15 days of PCI", which was the latest injection time analyzed, obtaining -0.71% (95% CI -4.90-3.48; $p = 0.74$). Thus, the meta-analysis suggests that the optimum injection time of BM-MNCs to improve LVEF levels after an acute myocardial infarction is within 24 hours of reperfusion.

After investigating the human body's natural mechanisms of wound healing following a heart attack and researching about the biological processes involved in tissue repair, some biological aspects that align with the results obtained from the meta-analysis were discovered. Through the research on this topic, it can be assumed that the possible biological explanation for those results is given by the fact that the activity of paracrine factors, which are produced by BM-MNCs, is intensified by the inflammatory process that takes place on the first days following reperfusion. The release of paracrine factors, such as cytokines and growth factors, directly influence myocardium repair through a number of restorative aspects, which include myocardial protection, neovascularization, cardiac remodeling, and differentiation (Mirotsou, et al., 2011).

Although similar studies have investigated the effects of timing in stem cell therapy, the relatively recent emergence of this concept and the great number of variables in this topic that could alter the results, makes this investigation a relevant research topic in this field of study. For instance, Xu et al. performed a similar investigation, in which the "between 7 and 10 days after PCI" group had the highest LVEF improvement. However, this study did not focus on investigating the effects of only BM-MNCs, instead it included a wide range of infused cells, such as isolated mesenchymal cells and progenitor cells, BM stromal cells or general BM cells. This fact could explain the disparity of results between the studies, due to the slightly distinct biological properties amongst the different cell types.

Overall, this study was able to answer the research question by performing a meta-analysis of the trials and investigating the biological aspects involved in the process. Nevertheless, the biggest struggle faced while writing this investigation was the lack of standardization between the trials analyzed, which is due to the many variables involved in this topic. For this reason, additional research is needed to determine the ideal conditions of the elements involved, such as the injected cell type or the number of cells infused, and how they affect the final results.

Limitations

Because of the lack of standardization between the trials, it was not possible to find studies with exactly the same design and methods, which led to variations between them. One example of this is the follow-up time. In some trials, the follow-up data is measured at 6 months after baseline and in others at 4 months or 3 months after baseline. This factor could result in a slight discrepancy between the results as the myocardium conditions can change in a matter of months. However, considering the limitations of trial availability, all the trials were included in order to analyze these specific time frames and obtain a larger sample size. To ensure greater reliability of this study, all the trials analyzed would need to have the same follow-up date. Correspondingly, the studies also presented different ways of measuring the LVEF levels, with some trials measuring by CMRI and others by Echocardiograms or LV Angiography. This can result in small variations in the LVEF levels between trials, which could impact the overall outcomes. Aiming to ensure data accuracy, the measuring methods should be standardized.

The LVEF measuring itself can also be considered a limiting factor as it was the only way to determine tissue regeneration by analyzing the improvement of heart function. However, this only considers one aspect of heart function and does not give a global perspective of the myocardium. In order to overcome this weakness of the study, it would be necessary to have a complete examination of myocardium improvement, considering not only LVEF levels but the systolic and diastolic functions as well as blood pressure. This research aims to investigate only the short-term effects of the treatment, with the longest follow-up analyzed being 6 months after baseline. With this being said, the results obtained with this investigation may change if analyzed from a perspective of 12 or 18 months after follow-up. The trials would need longer follow-up dates to determine the long-term implications of this therapy.

References

Assmus, B., Scha¨chingerV., Teupe, C., Britten, M., Lehmann, R., Do¨bertN., Gru¨nwaldF., Aicher, A., Urbich, C., Martin, H., Hoelzer, D., Dimmeler, S., & Zeiher, A. M. (2002). Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation*, *106*(24), 3009–3017. https://doi.org/10.1161/01.cir.0000043246.74879.cd

Caplan, A. I., & Dennis, J. E. (2006). Mesenchymal stem cells as trophic mediators. *Journal of Cellular Biochemistry*, *98*(5), 1076–1084. https://doi.org/10.1002/jcb.20886

Cleveland Clinic. (2019). *Heart Attack (Myocardial Infarction) | Cleveland Clinic*. Cleveland Clinic. https://my.clevelandclinic.org/health/diseases/16818-heart-attack-myocardial-infarction

Frangogiannis, N. (2002). The inflammatory response in myocardial infarction. *Cardiovascular Research*, *53*(1), 31–47. https://doi.org/10.1016/s0008-6363(01)00434-5

Gilbert, S. F. (2000). *Paracrine Factors*. http://www.ncbi.nlm.nih.gov/books/NBK10071/

Gnecchi, M., Zhang, Z., Ni, A., & Dzau, V. J. (2008). Paracrine Mechanisms in Adult Stem Cell Signaling and Therapy. *Circulation Research*, *103*(11), 1204–1219. https://doi.org/10.1161/circresaha.108.176826

Grajek, S., Popiel, M., Gil, L., Breborowicz, P., Lesiak, M., Czepczynski, R., Sawinski, K., Straburzynska-Migaj, E., Araszkiewicz, A., Czyz, A., Kozlowska-Skrzypczak, M., & Komarnicki, M. (2009). Influence of bone marrow stem cells on left ventricle perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: randomized clinical trial: Impact of bone marrow stem cell intracoronary infusion on improvement of microcirculation. *European Heart Journal*, *31*(6), 691–702. https://doi.org/10.1093/eurheartj/ehp536

Hodgkinson, C. P., Bareja, A., Gomez, J. A., & Dzau, V. J. (2016). Emerging Concepts in Paracrine Mechanisms in Regenerative Cardiovascular Medicine and Biology. *Circulation Research*, *118*(1), 95–107. https://doi.org/10.1161/circresaha.115.305373

Huang, R., Yao, K., Sun, A., Qian, J., Ge, L., Zhang, Y., Niu, Y., Wang, K., Zou, Y., & Ge, J. (2015). Timing for intracoronary administration of bone marrow mononuclear cells after acute ST-elevation myocardial infarction: a pilot study. *Stem Cell Research & Therapy*, *6*(1). https://doi.org/10.1186/s13287-015-0102-5

Jansen of Lorkeers, S. J., Eding, J. E. C., Vesterinen, H. M., van der Spoel, T. I. G., Sena, E. S., Duckers, H. J., Doevendans, P. A., Macleod, M. R., & Chamuleau, S. A. J. (2015). Similar Effect of Autologous and Allogeneic Cell Therapy for Ischemic Heart Disease. *Circulation Research*, *116*(1), 80–86. https://doi.org/10.1161/circresaha.116.304872

Janssens, S., Dubois, C., Bogaert, J., Theunissen, K., Deroose, C., Desmet, W., Kalantzi, M., Herbots, L., Sinnaeve, P., Dens, J., Maertens, J., Rademakers, F., Dymarkowski, S., Gheysens, O., Van Cleemput, J., Bormans, G., Nuyts, J., Belmans, A., Mortelmans, L., & Boogaerts, M. (2006). Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. *The Lancet*, *367*(9505), 113–121. https://doi.org/10.1016/S0140-6736(05)67861-0

Kikuchi, K., & Poss, K. D. (2012). Cardiac Regenerative Capacity and Mechanisms. *Annual Review of Cell and Developmental Biology*, *28*, 719–741. https://doi.org/10.1146/annurev-cellbio-101011-155739

Kikuchi-Taura, A., Okinaka, Y., Takeuchi, Y., Ogawa, Y., Maeda, M., Kataoka, Y., Yasui, T., Kimura, T., Gul, S., Claussen, C., Boltze, J., & Taguchi, A. (2020). Bone Marrow Mononuclear Cells Activate Angiogenesis via Gap Junction-Mediated Cell-Cell Interaction. *Stroke*, *51*(4), 1279–1289. https://doi.org/10.1161/STROKEAHA.119.028072

Kinnaird, T., Stabile, E., Burnett, M. S., Lee, C. W., Barr, S., Fuchs, S., & Epstein, S. E. (2004). Marrow-Derived Stromal Cells Express Genes Encoding a Broad Spectrum of Arteriogenic Cytokines and Promote In Vitro and In Vivo Arteriogenesis Through Paracrine Mechanisms. *Circulation Research*, *94*(5), 678–685. https://doi.org/10.1161/01.res.0000118601.37875.ac

Krantz, D. S. (1980). Cognitive Processes and Recovery from Heart Attack: A Review and Theoretical Analysis. *Journal of Human Stress*, *6*(3), 27–38. https://doi.org/10.1080/0097840x.1980.9936096

Kusuma, G. D., Carthew, J., Lim, R., & Frith, J. E. (2017). Effect of the Microenvironment on Mesenchymal Stem Cell Paracrine Signaling: Opportunities to Engineer the Therapeutic Effect. *Stem Cells and Development*, *26*(9), 617–631. https://doi.org/10.1089/scd.2016.0349

Mirotsou, M., Jayawardena, T. M., Schmeckpeper, J., Gnecchi, M., & Dzau, V. J. (2011). Paracrine mechanisms of stem cell reparative and regenerative actions in the heart. *Journal of Molecular and Cellular Cardiology*, *50*(2), 280–289. https://doi.org/10.1016/j.yjmcc.2010.08.005

Perin, E. C. (2016). Intravenous, Intracoronary, Transendocardial, and Advential Delivery. *Stem Cell and Gene Therapy for Cardiovascular Disease*, 279–287. https://doi.org/10.1016/b978-0-12-801888-0.00022-9

Peteiro, J., Peteiro-Vázquez, J., Gacía-Campos, A., García-Bueno, L., Abugattás-de-Torres, J. P., & Castro-Beiras, A. (2011). The causes, consequences, and treatment of left or right heart failure. *Vascular Health and Risk Management*, *7*, 237. https://doi.org/10.2147/vhrm.s10669

Renuka, S., & Sethu, G. (2015). Regeneration after Myocardial Infarction. *Research Journal of Pharmacy and Technology*, *8*(6), 738. https://doi.org/10.5958/0974-360x.2015.00117.1

Review Manager (RevMan) [Computer program]. Version 5.4. The Cochrane Collaboration, 2020.

Roncalli, J., Mouquet, F., Piot, C., Trochu, J.-N., Le Corvoisier, P., Neuder, Y., Le Tourneau, T., Agostini, D., Gaxotte, V., Sportouch, C., Galinier, M., Crochet, D., Teiger, E., Richard, M.-J., Polge, A.-S., Beregi, J.-P., Manrique, A., Carrie, D., Susen, S., & Klein, B. (2010). Intracoronary autologous mononucleated bone marrow cell infusion for acute myocardial infarction: results of the randomized multicenter BONAMI trial. *European Heart Journal*, *32*(14), 1748–1757. https://doi.org/10.1093/eurheartj/ehq455

Schächinger, V., Erbs, S., Elsässer, A., Haberbosch, W., Hambrecht, R., Hölschermann, H., Yu, J., Corti, R., Mathey, D. G., Hamm, C. W., Süselbeck, T., Assmus, B., Tonn, T., Dimmeler, S., & Zeiher, A. M. (2006). Intracoronary Bone Marrow–Derived Progenitor Cells in Acute Myocardial Infarction. *New England Journal of Medicine*, *355*(12), 1210–1221. https://doi.org/10.1056/nejmoa060186

Segers, V. F. M., & De Keulenaer, G. W. (2021). Autocrine Signaling in Cardiac Remodeling: A Rich Source of Therapeutic Targets. *Journal of the American Heart Association*, *10*(3). https://doi.org/10.1161/jaha.120.019169

Seropian, I. M., Toldo, S., Van Tassell, B. W., & Abbate, A. (2014). Anti-Inflammatory Strategies for Ventricular Remodeling Following ST-Segment Elevation Acute Myocardial Infarction. *Journal of the American College of Cardiology*, *63*(16), 1593–1603. https://doi.org/10.1016/j.jacc.2014.01.014

Sid-Otmane, C., Perrault, L. P., & Ly, H. Q. (2020). Mesenchymal stem cell mediates cardiac repair through autocrine, paracrine and endocrine axes. *Journal of Translational Medicine*, *18*(1). https://doi.org/10.1186/s12967-020-02504-8

Sürder, D., Manka, R., Lo Cicero, V., Moccetti, T., Rufibach, K., Soncin, S., Turchetto, L., Radrizzani, M., Astori, G., Schwitter, J., Erne, P., Zuber, M., Auf der Maur, C., Jamshidi, P., Gaemperli, O., Windecker, S., Moschovitis, A., Wahl, A., Bühler, I., & Wyss, C. (2013). Intracoronary Injection of Bone Marrow–Derived Mononuclear Cells Early or Late After Acute Myocardial Infarction. *Circulation*, *127*(19), 1968–1979. https://doi.org/10.1161/circulationaha.112.001035

Sutton, M. G. St. J., & Sharpe, N. (2000). Left Ventricular Remodeling After Myocardial Infarction. *Circulation*, *101*(25), 2981–2988. https://doi.org/10.1161/01.cir.101.25.2981

Tang, Y. L., Zhao, Q., Zhang, Y. C., Cheng, L., Liu, M., Shi, J., Yang, Y. Z., Pan, C., Ge, J., & Phillips, M. I. (2004). Autologous mesenchymal stem cell transplantation induce VEGF and neovascularization in ischemic myocardium. *Regulatory Peptides*, *117*(1), 3–10. https://doi.org/10.1016/j.regpep.2003.09.005

Traverse, J. H. (2011). Effect of Intracoronary Delivery of Autologous Bone Marrow Mononuclear Cells 2 to 3 Weeks Following Acute Myocardial Infarction on Left Ventricular Function. *JAMA*, *306*(19), 2110. https://doi.org/10.1001/jama.2011.1670

Wollert, K. C., Meyer, G. P., Müller-Ehmsen, J., Tschöpe, C., Bonarjee, V., Larsen, A. I., May, A. E., Empen, K., Chorianopoulos, E., Tebbe, U., Waltenberger, J., Mahrholdt, H., Ritter, B., Pirr, J., Fischer, D., Korf-Klingebiel, M., Arseniev, L., Heuft, H.-G., Brinchmann, J. E., & Messinger, D. (2017). Intracoronary autologous bone marrow cell transfer after myocardial infarction: the BOOST-2 randomised placebo-controlled clinical trial. *European Heart Journal*, *38*(39), 2936–2943. https://doi.org/10.1093/eurheartj/ehx188

World Health Organization (2022). "Cardiovascular Diseases." *World Health Organization.* www.who.int/health-topics/cardiovascular-diseases#tab=tab_1

Xu, J., Liu, D., Zhong, Y., & Huang, R. (2017). Effects of timing on intracoronary autologous bone marrow-derived cell transplantation in acute myocardial infarction: a meta-analysis of randomized controlled trials. *Stem Cell Research & Therapy*, *8*(1). https://doi.org/10.1186/s13287-017-0680-5