Hemostatic Status of Pre and Post Intracoronary Injection of Peripheral Blood Stem Cells in Patients with Recent Myocardial Infarction

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ABSTRAK

Tujuan: *untuk melaporkan perubahan-perubahan parameter hemostasis, misalnya agregasi trombosit, viskositas darah dan plasma, waktu protrombin, APTT, CRP, dan fibrinogen sebelum dan sesudah pemberian terapi sel punca.* ***Metode:*** *terdapat 24 pasien yang ikut serta dalam penelitian ini. PBSCs dipanen dan disuntikkan ke arteri yang berhubungan dengan infark (infarct-related artery) setelah pemberian G-CSF selama 5 hari berturut-turut. Rekombinan eritropoietin manusia/recombinant human erythropoietin diberikan pada saat penyuntikan PBSCs intrakoroner dilakukan.* ***Hasil:*** *kami dapat melakukan evaluasi pada 11 dari 24 pasien tentang status hemostasis pra- dan pasca-suntikan sel punca. Tidak ada perbedaan yang bermakna antara data dasar dan 3 bulan kemudian dalam hal aggregasi spontan (p=0,350), PT (p=0,793), aPTT (p=0,255) dan TT (p=0,254). Juga tidak ada perbedaan bermakna antara data dasar dan 3 bulan kemudian dalam hal viskositas plasma (p=0,442) dan viskositas darah (p=0,843). Meskipun demikian, pasien yang memiliki viskositas darah dan plasma di atas atau di bawah kisaran normal nilai laboratorium belum kembali ke titik normal setelah perlakuan. Baik PT maupun APTT, keduanya menunjukkan nilai normal. Kadar fibrinogen dan CRP menunjukkan penurunan yang bermakna antara data dasar dan 3 bulan setelah pengobatan, masing-masing dengan nilai p=0,009 dan p=0,04.* ***Kesimpulan:*** *kombinasi G-CSF dan EPO based-intracoronary infusion dari PBSCs dapat membuka perspektif baru dalam pengobatan keadaan hiperkoagulabilitas pasca infark miokardium akut/AMI.*

Kata kunci: *koagulasi, agregasi trombosit, infark miokardium, hiperkoagulasi.*

ABSTRACT

Aim: *to investigate hemostatic parameter changes, such as platelet aggregation, blood and plasma viscosity, prothrombin time, APTT, CRP and fibrinogen, before and after administration of stem cell therapy.* ***Methods:*** *a total of 24 patients were enrolled. Peripheral blood stem cells (PBSCs) were harvested and injected into the infarct-related artery after 5 consecutive days of G-CSF administration. Recombinant human erythropoietin was administered at the time of intracoronary PBSCs injection.* ***Results:*** *we were able to evaluate*

11 from 24 of patients regarding hemostatic status pre–post stem cell injection. There were no significant difference between baseline vs 3 months in spontaneous aggregation (p=0.350), PT (p=0.793), aPTT (p=0.255) and TT (p=0.254). There were also no significant difference between baseline vs 3 months in plasma viscosity (p=0.442) and blood viscosity (p=0.843). Nevertheless the patient who had their blood and plasma viscosity above or below normal laboratory range return to normal level after the treatment. Both PT and APTT also show normalization value. Both Fibrinogen and CRP level show significant decrease between baseline and 3 months after treatment (p=0.009) and (p=0.04) respectively. ***Conclusion:*** *combined G-CSF and EPO based-intracoronary infusion of PBSCs may open new perspective in the treatment of hypercoagulable state post AMI.*

Key words*: coagulation, platelet aggregation, myocardial infarction, hypercoagulation.*

INTRODUCTION

Cardiovascular disease has increased in the last half century, becoming one of the major causes of morbidity in all hospital admissions. Hypertension and coronary heart disease (CHD) constitute a major bulk of these CVD cases, where myocardial infarction (MI) constitutes half of all CHD cases.[1] In 2008, 1 of 6 deaths in United States was caused by coronary heart disease.[2] Current guidelines emphasize acute myocardial infarction (AMI) therapy on early coronary reperfusion that alleviates mortality rates such as primary percutaneous coronary intervention (PCI) or thrombolytic therapy. However, these conventional therapy sometimes cannot reverse the damage to infarcted myocardium.[1,3]

Virchow in 1856 postulated a triad of predisposition to thrombus formation. These triads are abnormalities of blood flow, blood constituents, and vessel wall. Abnormal elevation, even one of these factors, predispose to a "prothrombotic" or "hypercoagulable" state.[4,5] Population of patients with AMI showed these state very clearly. Even those undergoing optimal treatment procedure are still at risk for further ischemic events not only because procedure-related platelet activation occurs, but also due to the persistent platelet hyperreactivity and enhanced thrombin generation associated with ACS.[6,7] Hyperreactivity of platelets was shown in TIMI 12 trial up to 28 days and PREPARE trial in up to 6 months.[8-10] It is not known how the acute coronary thrombosis alter the kinetics of the systemic coagulation system. There are some theory proposed like increased activity of fIX and decreased fII and fV, elevated fVII antigen and coagulant activity.[11]

Latest researches has highlighted stem cell therapy for patient population at risk for heart failure. Recent meta-analysis showed adult stem cell have shown significant, safety and favourable impact in treating heart failure and myocardial infarction.[3,12-15] G-CSF-based stem cell therapy has been proposed as a practical and non-invasive alternative to stem cell therapy using bone marrow stem cells.[16] G-CSF might be considered mostly as a mobilizer to enrich peripheral blood stem cells (PBSCs). Despite the potential adverse effects of increasing vascular events, short term use of G-CSF in patients with AMI seems to be safe.

In 2008, we published an article showing 18 patients with anterior ST-segment elevation AMI, which showed improvement in most of the cardiac functions after injection of peripheral blood stem cells (PBSCs) into the infarct-related artery.[3] With the same protocol we are able to evaluate 6 more patients further regarding pre- and post- treatment (3 months) hemostatic profile such as platelet aggregation, blood and plasma viscosity, prothrombin time, APTT, CRP and fibrinogen. The purpose of this study is to investigate hemostasic parameter changes before and after administration of stem cell therapy.

METHODS

We enrolled 24 patients diagnosed with ST-segment elevation anterior wall infarction who had successful PCI with drug-eluting stent implantation and were referred late (more than 2 hours) to our hospital but still within 15 days after onset of symptom. None of the patients received fibrinolytic therapy. PCI was performed regardless of the patients' hemodynamic condition. Other

inclusion criteria were as follows: age 20 years or over and subject's willingness to comply with specific follow-up evaluations. All patients had complete revascularization even in non-infarct related territory. Exclusion criteria were: 1). hemodynamic instability during the procedure or any condition that may put the patient at undue risk such as pulmonary edema and cardiogenic shock, 2). previous or current severe co-existing diseases, such as cancer, hematological disorders (Hb <10 g/dL, WBC <4 or >11x109/L, or platelets <100x109/L), renal failure (creatinine level >2.5 mg/dL, or creatinine clearance <30 cc/min), hepatic dysfunction, serious infection or any co-morbidities that may impact patients' survival), 3). valvular heart disease and prosthetic valves, 4). hypertrophic or restrictive cardiomyopathy, 5). women of child bearing potential, and 6). lack of informed consent.

The study was conducted in accordance with the declaration of Helsinki and was approved by the University of Indonesia Medical School Ethical Committee. All patients gave written informed consent.

Study Protocol

One-to-three days after PCI, a daily dose of 10 µg/kg/day of G-CSF (Lenograstim [GranocyteTM], Aventis-Sanofi)) was administered subcutaneously for 5 consecutive days. CD34+ and CD 45+ cells were quantified at days 1 (baseline), 3, and 5 after injection and subsequently the peripheral blood stem cells (PBSCs) were harvested. A total of 150 ml of PBSCs was processed from the brachial vein using COBE Spectra and placed into a peripheral blood (PB) unfiltered collection bag (Baxter Health Care Corp, IL), which was discarded after PB infusion. CD34+ and CD 45+ cells enumeration was performed using FACSCalibur (Becton Dickinson).

To ensure sterility of the harvesting procedure, the white cell concentrate was cultured for bacteria and fungi. Three milliliters of the cell concentrate was injected into BacT Alert PF Pediatric tube (Biomerieux Inc, Durham, NC, cat No. 259794) and the incubated in the BacT Alert 120 system (Organon Teknika, Durham, NC) for 7 days or until the culture become positive. The cell concentrate was also cultured on Sabouraud agar medium (Oxoid, cat No. CM 0041) for fungi.

Patients were heparinized during stem cell injection. Cells were injected with the use of a stop flow technique through an over the wire balloon catheter positioned within the segment containing of the stent. The balloon was inflated with low pressure to completely block the blood flow for 3 min, during which time 5-6 ml PBSC suspension was injected. This was interrupted by 3 min of reflow by deflating the balloon to minimize the likelihood of extensive ischemia. After completion of intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency and absence of capillary plugging by measuring the myocardial blush grade. Approximately 15-25 x 106 PBSCs were injected during each procedure. All (except one) patients received subcutaneous 4000 IU of recombinant human erythropoietin (EprexTM) and glycoprotein IIb/IIIa inhibitors (IntergrellinTM) intravenous bolus and infusion at the time of intracoronary PBSCs injection. Patients were discharged the day after. Unless there was contraindication, all patients also received optimal standard therapy including aspirin, clopidogrel, nitrates, b-blockers, ACE-inhibitor/angiotensin-receptor blockers and statins.

The additional parameter compared in this study were PT, APTT, TT, platelet aggregation, blood and plasma viscosity, fibrinogen and CRP. All parameters were checked before and 3 months after treatment.

RESULTS

Almost all patients were male in gender and most of them were at the age of 50-59. The common ACS risk factors existed in the patient were dyslipidemia (52%), smoking (42%), and family history (25%).

Table 2 shows platelet aggregation from all patients before and after treatment of cell-therapy. No significant difference between baseline and 3 months after in spontaneous aggregation (3.11%$\pm$0.02% vs 5.39%$\pm$0.07%; p=0.350).

Table 1. Patient's characteristics

Criteria	n (%)
Age Group	
- 40-49	2 (8.0)
- 50-59	16 (67.0)
- 60-69	6 (25.0)
Sex	
- Male	23 (96.0)
- Female	1 (4.0)
History	
- Hypertension	4 (17.0)
- Diabetes	3 (13.0)
- Dyslipidemia	13 (54.0)
- Smoking	10 (42.0)
- Obesity	5 (21.0)
- Family	6 (25.0)
Blood test	
- Ureum >40 mg/dl (n=19)	5 (26.0)
- Creatinin >1.5 mg/dl (n=19)	0 (0)
- Total Cholesterol >200 mg/dl (n=18)	7 (39.0)
- HDL <50 mg/dl (n=18)	16 (89.0)
- LDL >150 mg/dl (n=18)	4 (22.0)
- Uric Acid >7 mg/dl (n=15)	5 (33.0)

No significant difference between baseline and after 3 months treatment in plasma viscosity (1.64±0.14% vs 1.63±0.07%; p=0.442) and blood viscosity (8.06 ±1.70 vs 7.56±0.83%; p=0.843). On the other hand fibrinogen and CRP level showed significant decrease between baseline and 3 months after treatment (575±165 vs 354±93; p=0.009) and (p=0.04) respectively.

Eventhough **Table 2** shows no significant changes in blood and plasma viscosity, **Figure 1** show trends of normalization. The patients who had their blood and plasma viscosity above normal range returned to normal point after the treatment. The same thing observed in patient who have their viscosity below normal range, it elevated to normal range.

No significant difference in: PT (12.31±0.80 vs 12.44±1.36; p=0.793), APTT (36.59±17.89 vs 30.25±2.52; p=0.255) and TT (15.9±0.33 vs 19.7 ±7.52; p=0.254) at baseline and at 3 months after therapy. Both PT and APTT also show normalization value, as seen in **Table 4**.

DISCUSSION

Stem cell therapy is an intervention strategy that introduces adult stem cells into damaged tissue in order to treat disease or injury. Whereas stem cells are biological cells found in all multicellular organisms that can divide and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. There are minimally 4 accessible source of adult stem cells in humans, bone marrow, adipose tissue, blood, and wharton jelly (umbilical cord).[17-20]

In this study we do an ad hoc analysis comparing hemostatic profile before and after injection of PBSC cell therapy. We emphasize the

Table 2. Platelet aggregattion at baseline and after 3 months treatment

No.	Baseline			3 Months After PSCT		
	Spont Aggr	Aggre 5	Aggre 10	Spont Aggr	Aggre 5	Aggre 10
1	3.20%	2.30%	2.70%	6.40%	12.30%	9.10%
2	1.80%	10.00%	12.70%	3.20%	25.50%	35.90%
3	7.30%	10.00%	10.90%	4.10%	1.40%	4.50%
4	4.50%	4.50%	11.40%	4.10%	14.50%	13.20%
5	3.20%	10.90%	17.70%	3.60%	9.10%	20.50%
6	4.10%	57.30%	62.70%	0.90%	36.80%	47.30%
7	3.20%	21.40%	26.40%	1.40%	34.60%	45.00%
8	1.40%	50.90%	55.90%	3.60%	20.00%	32.30%
9	3.20%	8.60%	13.60%	5.60%	3.20%	12.90%
10	2.70%	19.80%	27.30%	1.10%	9.70%	15.00%
11	2.20%	5.80%	65.80%	3.10%	6.00%	8.00%
Mean±SD	3.35±0.02	18.32±0.10	27.92±0.23	3.37±0.02	15.74±0.12	22.15±0.15
p value				0.969	0.570	0.408

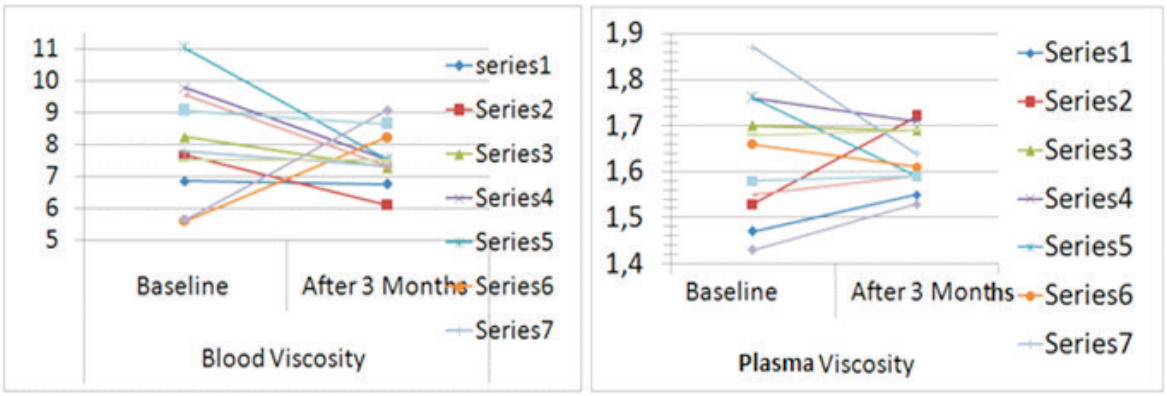

Figure 1. Normalization of blood and plasma viscosity at baseline and after 3 months of treatment

Table 3. Blood and plasma viscosity at baseline and after 3 months of therapy

No	Blood Viscosity		Plasma Viscosity		Fibrinogen (mg/dl)		CRP	
	Baseline	After 3 Months	Baseline	After 3 Months	Baseline	After 3 months	Baseline	After 3 months
1	6.85	6.74	1.47	1.55	377	314	20,1	4
2	7.66	6.10	1.53	1.72	937	352	6,9	17,1
3	8.24	7.26	1.70	1.69	458	454	103,1	0,01
4	9.77	7.49	1.76	1.71	680	282	40,4	20,1
5	11.02	7.52	1.76	1.59	552	321	85,3	0,8
6	5.60	8.24	1.66	1.61	446	352	1,5	0,01
7	7.77	7.32	1.87	1.64	570	504	20	1,86
8	9.54	7.31	1.55	1.59	478	468	53,68	6,05
9	7.50	7.50	1.68	1.69	726	218	8,47	13,92
10	5.64	9.07	1.43	1.53	530	276	191,94	1,63
11	9.05	8.63	1.58	1.59	575.4	354.1	53.139	6.548
mean±SD	8.06±1.70	7.56±0.83	1.64±0.14	1.63±0.07				

Table 4. PT-APTT-TT at baseline dan 3 months after treatment

PT		APTT		TT		
Baseline	After 3 Months	Baseline	After 3 Months	No of patients	Baseline	After 3 months
12.4	11.5	25.4	29.5	1	15.4	16.1
13.4	11.9	89.9	32.1	2	15.9	16.6
10.9	11.3	29.7	28.2	3	16.1	19.3
13.3	12.2	32.1	29.6	4	15.8	16.6
11.9	12.5	29.8	31.6	5	15.9	15.0
13.0	12.1	31.7	28.9	6	16.4	34.8
11.3	12.0	32.2	26.7	Mean±SD	15.9±0.33	19.7±7.52
12.3	11.9	32.8	31.3			
12.3	12.9	36.9	31.2			
12.3	16.1	30.6	35.8			
		31.4	27.8			

use of G-CSF as a mobilizer to enrich PBSCs. From 24 patients, most of them are males. The 3 most common risk factors associated with ACS are dyslipidemia, smoking, and family history which exist in 54%, 42%, and 25% of the patients. All patients in this study receive heparin until 3 days after intervention, and afterwards the treatments are continued with standard optimal therapy as indicated.

From 24 patients, we are able to follow 11 patients regarding the hemostatic profile. As shown in table 2-4 there are no significant difference between platelet aggregation, blood and plasma viscosity, PT, APTT, and TT pre- and post- treatment. However, surprising result can be seen in **Figure 1**. There are 8 patients who have increased blood viscosity and 3 patients with increased plasma viscosity before treatment who return to normal laboratory range after treatment. On the other side, there are 2 patients with decreased blood viscosity and 4 patients who have decreased plasma viscosity before treatment. And after treatment they have their laboratory result elevated to normal range. This event have never been reported before, and we refer this event to "normalization". Moreover, platelet aggregation, PT, APTT, and TT all also showed this normalization. These result does not attributable to anticoagulant therapy, because all patient receive heparin only until the third day post-PCI.

According to some studies, restenosis post PCI in AMI patients occurs in 10-60% cases.[21] There are several proposed mechanism as to why these event could happen. First, mechanical disruption of coronary plagues by denudation of the arterial endothelium and subsequent exposure to thrombogenic matrix protein, in addition to underlying atherothrombotic disease, results in platelet activation during or after PCI.[6] Second, it was found that ACS was associated with persistent platelet hyperreactivity and enhanced thrombin generation. In the thrombolysis in myocardial infarction (TIMI) 12 trials, activation of platelets continues even until 28 days after clinical stabilization post-ACS.[6,10] Another study, the platelet reactivity in patients and recurrent events (PREPARE) POST-STENTING study also demonstrated higher posttreatment ADP-induced aggregation in patients who suffered ischemic events over a 6-month period compared with patients without ischemic events (p=0.02).[6]

There is also reported that the severity of thrombosis was related with more severe or higher level of prothrombotic state as well as reactivity of the platelets.

Other interesting parameter are fibrinogen and CRP, both are acute phase markers, showed significant decrease before and after treatment. A research showed that an increase in 1 g/L of plasma fibrinogen was associated with a 45% increased risk of MI.[22] CRP is an indicator of the severity of STEMI and the occurence of complications during hospitalization.[23] It is also independently stratify patients for in-hospital mortality risk.[24] In 2005, Feinbloom suggested new emerging risk factors for arterial thrombosis, and among them are elevated CRP and fibrinogen.[25] The 2008 JUPITER trial demonstrates that there was another potential mechanism that increases arterial trombotic event among healthy individuals with elevated hsCRP. Recently, there is also an ongoing trial called Cardiovascular Inflammation Reduction Trial (CIRT), which main purpose is to confirm the inflammatory hypotesis of atherothrombosis, which include decreasing CRP level.[26] All these parameter changes are in contrast with other research which show that there are no changes in hemostatic profile after optimal treatments.[6,11]

The mechanism of normalization and significant decrease of fibrinogen and CRP is still unknown. But it may be related to how the stem cells works, such as paracrine effect and wound healing. The paracrine effect of stem cells has been reported to influence cardiac repair by protecting cardiac myocites from apoptotic stimuli or activate cardiac-resident stem cell to enhance endogenous repair capacity.[17] Another potential mechanism is that transplanted stem cell may secrete a variety of growth factor and cytokines, which in turn alter the systemic hemostatic profile.[27]

There were 3 limitations that need to be addresed regarding the present study. The first limitation concerns the size of the sample. The second limitation has to do with patient's baseline hemostasis profile. And lastly, the

third limitation there were no control group. Therefore further study is needed to ensure the underlying mechanism of this event. If future researches are able to conclude the mechanism underlying normalization of hemostatic profile after cell-therapy injection post-AMI, it will help in developing new strategies in the treatment of cardiovascular diseases. We also propose the use of TEG platelet mapping in patients after PCI treatment, to evaluate the complex interaction between fibrin, thrombin, platelets, and coagulation factors during a state of hypercoagulability, which will allow individualized therapy.[28]

CONCLUSION

Our study showed that 3 months after injection of intracoronary peripheral blood stem cell, all the parameters of the hemostatic become normal. Moreover, 2 parameters, fibrinogen and CRP both showed significant improvement. Future prospective studies are needed to investigate the underlying mechanism of this event.

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