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Potential Breakthrough for Treating Severe Coronavirus Disease-2019 (COVID-19) Patients in Intensive Care Unit: The Use of Autologous Activated Platelet-rich Plasma in Serial Cases of Indonesian Patients

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Abstract— *Purpose*: To evaluate the potential of autologous activated platelet-rich plasma (aaPRP) and the outcomes for treating severe Coronavirus Disease-2019 (COVID-19) patients in Intensive Care Unit (ICU).*Materials and methods*: A case series of four patients from Koja Regional Public Hospital (Koja RPH) whom admitted to the ICU due to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. *Results*: All patients had the initial symptoms of SARS-Cov-2 infection and became worse overtime, so the patients were admitted to ICU. Dyspnoea with low oxygen saturation was observed in all cases. All patients had comorbids for COVID-19. Severe COVID-19 was observed in all of patients, as they had increasing CRP level and low level of oxygen saturation. The outcome of this case study showed potential effectiveness of aaPRP as adjunctive severe COVID-19 treatment. *Conclusion*: Autologous activated PRP treatment could be feasible to apply in severe COVID-19 management.

Keywords— SARS-CoV-2, COVID-19, autologous, activated platelet-rich plasma, ARDS

1. Introduction

The positive cases of coronavirus disease-2019 (COVID-19) in Indonesia has been increasing rapidly since the first case found in March 2020 to date [1]. Indonesia has the fourth-highest weekly percentage increase amongst other South-East Asian countries [2]. Based on World Health Organization South-East Asia Region Office (WHO SEARO) data, Indonesia reported the third-highest death rate. Although most patients (81%) that contracted COVID-19 had mild symptoms, there were about 14% COVID-19 positive patients who had severe symptoms. Thus, they required hospitalization and oxygen support, and around 5% were admitted to the intensive care unit (ICU) [3].

Amongst other symptoms, cytokine storm is typical in patients admitted to ICU. Coronavirus 2 (SARS-CoV-2) virus disrupts human normal immune system resulting in uncontrolled inflammatory response [4]. Lympophenia, activation and lymphocyte dysfunction, granulocytes and monocytes abnormalities, and increased production of proinflammatory cytokines are some characteristics of cytokine storm or cytokine release syndrome (CRS) [4]. These conditions are associated with acute respiratory distress syndrome (ARDS), multiple organ failure, and most of death cases of COVID-19 [4,5].

An increase of inflammatory cytokines, such as interleukin 1 beta (IL-1β), IL-2, IL-6, and tumor necrosis factor alfa (TNF-α) is a hallmark of CRS [6,7]. There have been plenty of modalities investigated to find the right medication for CRS management in COVID-19 patients. Based on our research and experience in doing cell therapy for 9 years that were supported by many literatures [8,9,10], activated platelet-rich plasma (PRP) produces anti-inflammatory effects in inflammatory condition that is beneficial for tissue

regeneration. PRP decreases IL-1β, IL-6, IL-8, and TNFα inflammatory genes expression while also reduces IL-1β and TNFα inflammatory cytokines production in nucleus pulposus-derived stem cells from early inflamed degenerated intervertebral discs [10]. PRP has also been showed to contain interleukin 1 receptor antagonist (IL-1RA)[11], an anti-inflammatory cytokines that suppress IL-6 secretion [12].

Anti-inflammatory potency of PRP has been showed in autoimmunity diseases, such as rheumathoid arthritis [13] and chronic inflammatory diseases, such as diabetic foot ulcer [14] and degenerative disc disease [14,15]. Thus, we suggest that PRP treatment may posses some beneficial effect in CRS management in COVID-19 patients that should be further investigated. In this article, we reported serial clinical case regarding the use of PRP as adjuvant therapy to standard therapy for severe COVID-19 in four patients of Koja Regional Public Hospital (Koja RPH), Jakarta, Indonesia.

2. Case Series

2.1 Case #1

An obese smoker man (35 years) with history of chronic heart failure (CHF) presented to Koja Regional Public Hospital with fever, dyspnoea, and diarrhea for one week before admission. The symptoms started after patient had direct contact with his family member that tested positive for SARS-CoV-2. Previous haematology analysis before admission into hospital showed that red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and hematocrit (Ht) were within normal range with a reduced platelet count. Differential blood count showed basophils, eosinophils, neutrophils, and lymphocyte were all below the normal range. Patient received no medication prior to admission. Later, the rapid test and swab test result showed that this patient was positive with SARS-CoV-2. The patient was promptly admitted to emergency room in Koja RPH on September 7, 2020.

Chest x-ray scan revealed infiltration in both sides (left and right) of paracardial and suprahiller of the lung diagnosed with pneumonia (Figure 1). On day 0, respiration rate (RR) was increased to 28 times per minute, body temperature was 38.4°C, and heart rate (HR) was 110 times per minute. Oxygen saturation on 4 litres per minute (lpm) of O2 delivered by nasal cannula was 95%. RBC, WBC, Hb, Ht and platelet counts were within normal range. Differential blood count revealed that basophils, eosinophils, and lymphocytes were below the normal range, while neutrophils was above the normal range. This patient had low partial pressure of carbon dioxide (pCO_2) (20.1 mmHg) and low bicarbonate (HCO_3) (15 mEg/L) followed by metabolic acidosis with base excess -8.7 mEq/L (Table 1). Meanwhile, arterial blood gas (ABG) pH was 7.477 (normal range 7.350−7.450) indicated compensatory mechanism. Low blood sodium level was also observed with high Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) level. Patient had NaCl solution infusion and was given ceftriaxone (1x2 gr), azithromycin tablets (1x500 mg) for 5 days, oseltamivir (2x75 mg) for 5 days, ambroxol, cetirizine (2x1 tablet), vitamin C (3x1 tablet), and zinc (2x1 tablet). Patient was then transferred to the ward.

On day 1, D-dimer of this patient was very high (3038 ng/mL, normal range < 500 ng/mL) with prothrombin time (PT) 10.6 seconds (normal range 9.9 − 11.8 seconds) dan activated partial thromboplastin time (APTT) 30 seconds (normal range 31.00 − 47.00 seconds). Patient was given heparin 2x5000 IU by subcutaneous injection.

On day 2, the patient's condition was worsened. Sodium and potassium blood levels were below normal range. Oxygen saturation was 87% with increase RR (26 times per minute) and HR was 100 times per minute. The patient received 10 lpm O2 using non-rebreathing oxygen face mask (NRM). Oxygen saturation

level was still low (93.9%) and the rest of ABG values were still below normal range (pO_2, pCO_2, HCO_3, and base excess). O2 on NRM was them increased to 15 lpm. To increase airflow to the lung, patient also was given bronchodilator, aminophylline 480 mg/24 hours. The laboratory result (the P/F ratio was 70) revealed that patient had acute respiratory distress syndrome (ARDS). A nasopharyngeal swab PCR was positive to SARS-CoV-2.

On day 3, patient was hypoxemic, so oxygen therapy was delivered using high-flow nasal cannula (HFNC). On day 4, the oxygen saturation was within the normal range (94.5%). However, his condition had worsened and ineffective airway clearance was diagnosed. ABG values of pCO2, pO2, HCO3, and base excess were still below the normal range. On day 5, the patient was transferred to the intensive care unit (ICU) due to impending respiratory failure with worsening ARDS (the oxygen saturation was 89%). Post oxygen therapy with HFNC, oxygen saturation had increased to 97%. Aminophylline was administered with N-acetylsystein (NAC, 1200 mg) in 100 mL of normal saline (NS) for 4 hours per a day for 5 days. The patient received meropenem (3x1 gr for 24 hours) and was placed in prone position three times a day.

On day 7, he had low pCO_2 (30 mmHg) and pO_2 (62.7 mmHg) with unstable oxygen saturation (<88%). The patient had worsening dyspnoea (RR 48 times per minute) anddid not respond to HFNC, so the patient received intubation and was put into ventilator. Aminophylline was stopped. The sedation was administered (DOR 3 mg/hour) and 1000 mL of NS per 24 hours. ABG analysis was performed before and after 1 hour on ventilator. pCO_2 increased from 30.2 mmHg to 39.7 mmHg (normal range 32.00 – 45.00 mmHg), pO_2 increased from 62.5 mmHg to 176.5 mmHg (normal range 95.00 – 100.00 mmHg), and oxygen saturation increased from 92.4% to 99.8%. RR was reduced to 25 times per minute. The patient's chest x-ray showed that pneumonia was worsened with thicker infiltration in paracardial and suprahiller of the lung.

On day 8 (day 2 on ventilator), patient's breath control was adequate, GCS value was E4M6VETT. However, the patient still had respiratory distress. Swab test result was still positive with very high D-dimer value (9,97ng/mL). Activated autologous platelet-rich plasma (aaPRP) was administered. Nine hours after aaPRP was administered, body temperature was reduced from 38.2°C to 36.5°C, pulse was 114−115 times per minute, RR was 21 times per minute, blood pressure (BP) was reduced to 117/75 mmHg from 125/75 mmHg, oxygen saturation was 97% increase (before was 91%).

On day 9, swab test result was negative to SARS-CoV-2, target oxygen saturation and target ABG value were achieved, except pO_2 was 105.2 mmHg which was slightly above the normal range (95.0 – 100.00 mmHg). However, the patient had worsening dyspnoea with RR of 44 – 54 times per minute and hypertension (BP 164/108 mmHg, HR 137 times per minute). Acute kidney injury was diagnosed as urine output was less than 0.5 mL/kg/h. Chest X-ray showed acute lung edema. Compared to haematology test results on day 0, leucocytes, platelets, and neutrophils counts were all increased, while hematocrit and lymphocytes counts were decreased. PT, APTT, sodium, potassium, and chloride blood level were in normal range. SGOT was significantly reduced from 105 U/L to 22 U/L (normal range < 40 U/L) and SGPT was reduced from 71 U/L to 64 U/L (normal range < 41 U/L). Procalcitonin (PCT) value was less than 0.4 ng/mL (low risk of severe sepsis/septic shock < 0.5 ng/mL) and C-reactive protein (CRP) was 2.94 mg/dL (normal range < 0.50 ng/mL). For acute kidney injury management, patient was given lasix intravenously (40 mg), followed by lasix drip (120 mg) for 12 hours. The patient was also given Cedocard 2 mg/hours and uptitrated to 4 mg/hour to lower the blood pressure. Heparin was still administered with dosage of 15,000 IU uptitrated to 20,000 IU per 24 hours. Two hours post medication, blood pressure was reduced to 120/80 mmHg, HR and RR were reduced to 120 and 20 times per minute, oxygen saturation was increased to 96% with urine output increased to 1.6 mL/kg/h. Dexamethasone 3x1 ampoule were given intravenously. As the

patient had worsening dyspnoea, hethen received the second administration of aaPRP. Blood was taken for aaPRP processing contained platelets with concentration above the normal range (438 x 10^3/µL).

On day 10, patient still needed ventilator support. However, the target of oxygen saturation of 96% was achieved, dyspnoea was improved, lymphocyte count had increased from 3.0% to 3.6% and monocyte was close to normal range. Platelets count was reduced to 377 x 10^3/µL and to within the normal range (210 x 10^3/µL). On day 11, body temperature was increased to 37°C with increasing CRP value to 17.30 ng/mL from 6.84 ng/mL on day 10. The leucocytes count was lower than result on day 10, but still above the normal range (15.73 x 10^3/µL). Chest x-ray showed a worsening condition compared to day 7. Dexamethasone 2x1 ampoules was administered with IVFD, but azithromycin was stopped.

On day 12, dyspnoea was on and off with an increase of leucocytes count to 18.6 x 10^3 /µL. CRP was not checked. IVFD and heparin (20,000 IU) was still given, but dexamethasone was stopped. The third aaPRP was decided to be administered. Blood for this aaPRP contained normal platelet counts per µL (212 x 10^3/µL). After receiving aaPRP, oxygen saturation was increased to 99%. On day 14, patient had a significantly improvement of breathing and reduced dyspnoea with improvement of chest x-ray profile compared to day 11. Patient weaned from the ventilator and extubated. Postextubation, patient received 15 lpm O2 by NRM. A nasopharyngeal swab PCR test result was also negative of SARS-CoV-2. On day 17, neutrophils count was reduced to 88.8% and CRP concentration was reduced to 0.97, which was almost close to normal range (< 0.50 mg/dL). Chest x-ray on day 18 showed an improvement with a significant reduction of lung infiltration. On day 21, patient did not report any discomfort, so he was transferred to the ward and was discharged on day 23.

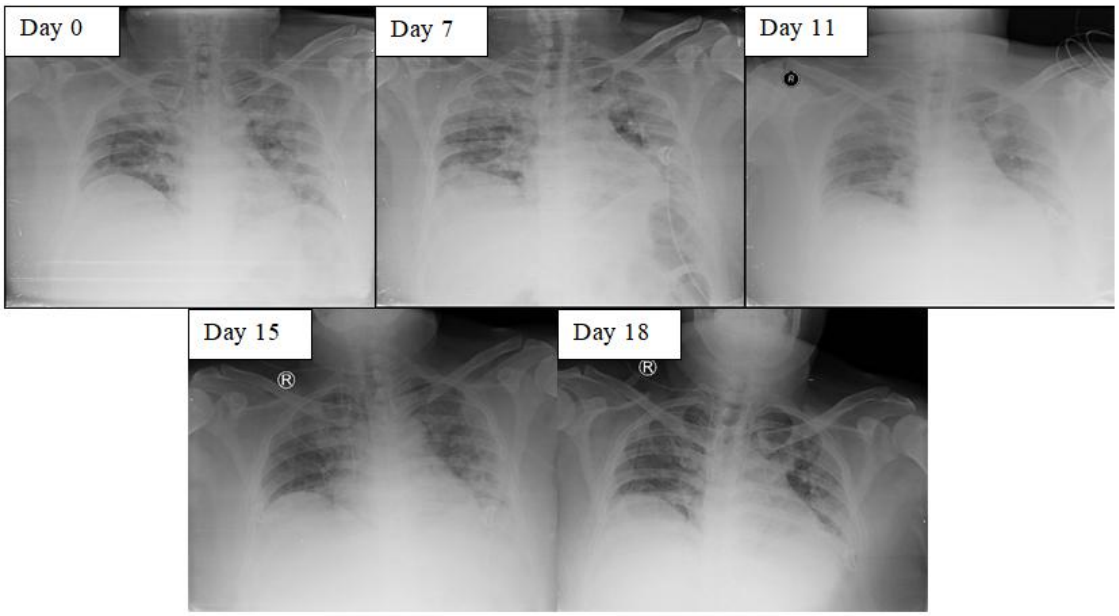

Figure 1. Serial chest X-ray.On going improvements on day 15 and 18 after three times of aaPRP treatment compared to day 0, 7 and 11.

Table 1. Laboratory parameters result of patient 1

	Day													
	0	1	2	3	4	7	8	9	10	11	12	14	18	19
Hematology														
Hemoglobin (12.5 - 16.0 g/dL)	15.8	**16.2**						14.1	13.6	12.6*	12.4*		12.5	12.9
Leukocytes (4.00 - 10.50 x 10^3/uL)	5.37	7.9						14.20	23.29	15.73	18.66		**16.37**	**16.77**
Hematocrit (37.0 - 47.0 %)	**44.5**	**45.2**						**40.8**	39.5	37.3	36.2*		35.5*	36.8*
Trombocyte (182 - 369 x 10^3/uL)	192	235						**438**	**377**	210	212		329	347
Erythrocyte (4.20 -5.40 x 10^6/uL)	**5.66**	**5.85**						4.97	4.89	4.52			4.49	4.62
Basophil (0.1 - 1.2%)	0.2	0.1						0.1	0.1	0.1			0.2	0.3
Eosinophil (0.7 - 5.8%)	0	0						0	0	0			0.0*	0.1*
Neutrophil (34.0 - 71.1%)	76.7*	80.1*						91.5	91.3	91.3			**92**	**88.8**
Lymphocyte (19.3 - 51.7%)	16.8*	13.3*						3.0"	3.6*	4.3*			4.5*	6.6*
Monocyte (4.7 - 12.5%)	6.3	6.5						5.4	5.0	4.3*			3.3*	4.2*
Arterial Blood Gas (ABG)														
pH (7.350 - 7.450)	**7.477**		7.399		7.448	**7.499**		7.391	7.415			7.426		
PCO2 (32.0 - 45.0 mmHg)	20.1*		29.2*		28.7*	30.0*		39	46.0			38.6		
PO2 (95.0 - 100.0 mmHg)	**110.1**		70.4*		70.2*	62.7*		**105.2**	96.0			167.0		
HCO3 (21.0 - 28.8 mEq/L)	15.0*		18.2*		20.0*	23.5		23.9	**29.7**			25.6		
Base Excess (-2.5 - +2.5)	-8.7*		-6.8*		-4.2*	0.1		-1.3	**4.9**			1.0		
O2 Saturation (94.00 - 100.00) %	98.9		93.9*		94.5	92.4*		97.6	96.6			99.4		
C-reactive protein (< 0.50 mg/dL)								**2.94**	**6.84**	**17.30**			**2.02**	**0.94**
Covid-19 Test	+		+	+			+		-			-		

Bold : highest than references
Asterisk (*) : lowest than references

2.2 Case #2

A male obese patient with history of type 2 diabetes mellitus, pulmonary tuberculosis, and coronary arterial disease (CAD) presented to Koja RPH with coughs, dyspnoea, and weakness. Hematological test was performed and showed low Hb (12.1 g/dl), leukocytosis (15.67 x 10^3/µl), low hematocrit (34.1 %), high thrombocyte count (408 x 10^3/µl), and low erythrocyte count (4.27 x 10^6/µl). Differential blood count showed dominance of neutrophils above its normal range (82.6%), decrease of lymphocytes below its normal range (11.9%), decrease of monocytes below its normal range (5%), and decrease of eosinophils (0.2%). Abnormal ABG was also observed for pH, PCO_2, PO_2, HCO_3, and base excess. High increase of CRP (23 mg/dl) and ESR (114 mm/hour) was also observed. The patient was later found to be positive in swab test for SARS-CoV-2 and hospitalized in the ward room for two days. Medications were dexamethasone 1x5 mg/IV, avigan 2x1.600 mg for a day and 2x600 mg for five consecutive days, ceftriaxone injection 1x2 g/IV for 5 days and then switched to oral tablet cefixime 2x200 mg and also subcutaneous heparin 1x5000 IU was given.

After 2 days, the patient was transferred to the intensive care unit (ICU) due to impending respiratory failure with worsening ARDS (the oxygen saturation was 86.9%). Medication was continued along with vitamin C 2x500 mg drip in 100 cc NaCl within 1 hour, hidonac 1200 mg in 100 cc NaCl within 4 hours for 5 days. Patient was administered first aaPRP on day 2 of ICU admission. On day 3, dyspnoea was improved and hematologic evaluation was performed. An increase of hemoglobin (13.3g/dL) compared to the first day was observed although still below normal range. Increase of thrombocyte count compared to the first day was also observed (513 x 10^3/µL). Further leukocytosis was observed (16.99 x 10^3/µL) with neutrophil dominance (90.6%) and decrease of lymphocytes (6.4%), monocytes (2.6%), and eosinophils (0.2%). CRP (4.52 mg/dl) and ESR (102 mm/hour, normal is 0−10 mm/hour) were still high but lower than on the day of

admission.

On day 4, further hematologic evaluation was done and patient showed similar results. However, further increase in leukocytosis was found (20.47 x 10^3/µl) with similar differential blood count). CRP (6.47 mg/dl) increased than the third day, while ESR (100 mm/hour) keeps decreasing.On day 5, the second aaPRP was administered to the patient and the patient was evaluated on the next day. Hematologic evaluation showed improving results and showing trend of returning to normal. Differential blood counts were also improving with reduced neutrophil dominance (86.4%).

On day 6, patient was placed on NMR 15 lpm. ABG still showed abnormal findings. By day 7, patient was also evaluated for ABG and still showed abnormal findings. Oxygen saturation on NMR 15 lpm was only 80%. NRM was then replaced with HFNC 45 lpm, but oxygen saturation only reached 90%. Patient was then administered thirdaaPRP. Vitamin C was switched to oral tablet 3x250 mg, and NAC was switched to oral tablet 3x400 mg.On day 8, patient was evaluated for ABG and showed improving results. Improving results was found in hematologic testing and showed trend of returning to normal values although leukocytosis (13.56 x 10^3/µl) and below normal value hematocrit (41%) was still observed.

On day 9, oxygen 45 lpm HFNC was changed to 40 lpm for 2 hours. ABG test was performed and showed abnormal findings with increased pH and base excess, but oxygen saturation increased to 98.1%. pactient was then placed on NRM 15 lpm.By day 10 on ICU, patient's dyspnoea had improved but still receiving NRM 15 lpm. By day 13, patient was transferred to ward room, had no discomfort until he was discharged on day 24.Chest x-ray scan was done on the day of admission, day 6, day 12, and day 16. Better chest x-ray scan findings was observed with lesser opacity in right supra-parahilar-paracardial and left parahilar-paracardial. Widening of hilus was not found. Right and left costophrenic sinus was sharp. All bones were intact. No new lesions were observed and heart was found to be big and normal.

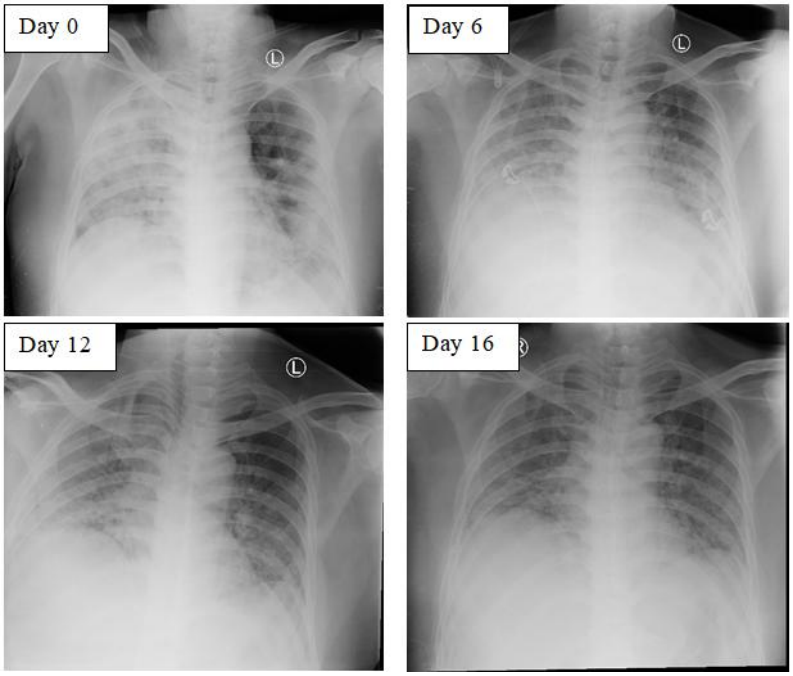

Figure 2. Serial chest x-ray showed lesser opacity in right supra-parahilar-paracardial and left parahilar-paracardial on day 12 after receiving three times aaPRP treatments

2.3 Case #3

A 52 year-old male obese patient presented to Koja RPH with dyspnoea, cough, influenza, malaise, epigastric pain, and headache. Patient had a history of direct contact with SARS-CoV-2 positive patient. An increase of CRP level up to 5.79 mg/dl was found (normal is < 0.5 mg/dL). The leukocyte count was normal, but differential blood count showed a slight increase of neutrophil (70.6%) and lymphocyte (20.6%) was observed. A slight decrease in thrombocyte (161.000/µL) count was also observed. One day after admission, patient was confirmed positive for SARS-CoV-2 by rapid test and swab test. Patient was then admitted to emergency room and received 12 lpm O_2 using NRM.

ABG (PCO2, PO2, HCO3, base excess) was found below normal range. Patient's O_2 saturation was still within normal range. However, chest x-ray scan revealed signs of ARDS. Patient had NaCl solution infusion of granisetron 1x4 mg, ketorolac 30 mg, omeprazole 40 mg and dexamethasone 1x5 mg with oral NAC 3x400 mg, vit C 3x250 mg, zinc 2x20 mg, azitromicin 1x500 mg per day for 5 days. On the same day, the condition was worsened, patient fell in critical condition and immediately transferred to the ICU in the next day.

On day 1 in ICU, patient was administered aaPRP. Further evaluation on patient was done and vital signs were still normal. On day 2, ineffective breathing pattern was still observed and hematology testing showed similar results. Interestingly, a decrease of CRP was observed although still above normal range (from 5.66 mg/dL to 1.84 mg/dL). On day 3, further swab test was done and yielded positive result for SARS-CoV-2. Patient was administered second aaPRP and also given heparin 1x5000 UI and avigan. Evaluation on patient showed less dyspnoea and ineffective breathing pattern.

On day 5, patient was administered the third aaPRP. On evaluation, patient's vital signs were normal, less dyspnoea and coughs. The day after swab test was done on patient and showed negative result for SARS-CoV-2. Patient's vital signs were normal with increased hematocrit (41.2%) and decreased ESR (68 mm/hour). Differential blood count showed trend of going back to normal with neutrophil decreasing (76.8%), lymphocytes increasing (13.9%), and eosinophils increasing (0.3%) to its normal range. CRP was also found to decrease (1.98 mg/dl).Patient was further evaluated and showed better symptoms from day 7-10. Patient was then discharged on day 10 with no discomfort.

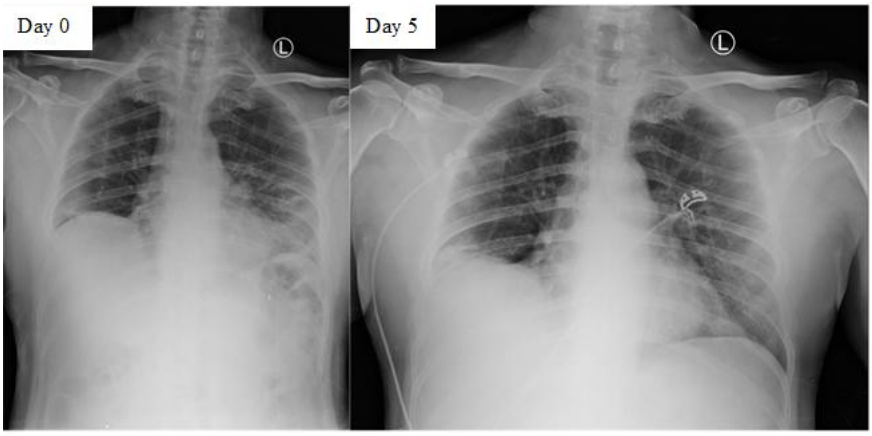

Figure 3. Serial chest x-ray showed improvements on day 5 after receiving two times aaPRP treatments

2.4 Case #4

A male obese patient presented to Koja RPH with diarrhea and coughs. Hematological testing showed normal results. However, differential blood count showed a sligh increase in neutrophils (69.5%). An increase in CRP was also observed (1.53 mg/dl). The result of swab test for SARS-CoV-2 was positive. Chest x-ray scan revealed signs of ARDS. Patient had IVFD RL 55 ml/12 hours, ceftriaxone injection 1x2

g/IV for 5 days and switched to oral tablet cefixime 2x200 mg, azithromycin tablet 1x500 mg for 5 days, and oseltamivir 2x75 mg for 5 days.

Patient was then admitted to the ward room. Patient was placed on NRM 4 lpm. On day 7, hypertension and disseminated intravascular coagulation was observed, with was still positive for SARS-CoV-2 . Increase of RR (33 times per minute) and a decrease of oxygen saturation to 83% was also found. Nasal canula 4 lpm was changed to 15 lpm O_2 NRM. An increase of CRP (9.48 mg/dl) was also observed. Patient was given dexamethasone 1x5 mg/IV and avigan 2x1.600 mg for a day and 2x600 mg for five consecutive days. Due to these worsening conditions, he was transferred to the ICU.

On day 1 in the ICU, ineffective airway clearance was observed. RR reached 30 times per minute and oxygen saturation was still low (92%) on NRM 15 lpm. ABG showed values was not within normal range with an increase of pH (7.48), a decrease of PO2 up to 30.8 mmHg, an increase of base excess (4.4). The dexamethasone and avigan medications were continued and patient was given meropenem 3x1 gram, hidonac 1200 mg in 100 cc NaCl within 4 hours for 5 days, subcutaneous heparin 1x5000 IU, and 6x200 cc NGT administrations.

Due to worsening symptoms on day 3 and high CRP (7.09 mg/dL), patient was administered aaPRP. On day 4, improvement of dyspnoea was observed with decreasing of CRP to 3.71 mg/dL. On day 5, the second aaPRP was administered following improvement of respiration and decreasing CRP to 1.17 mg/dL. I then revealed that swab result was negative for SARS-CoV-2. On day 7, the third aaPRP was administered and the day after, patient showed minimal symptoms and transferred to the ward room. Patient was released from hospital four days after.

3. Discussion

This report described the progression of four positive severe COVID-19 patientswith resolved ARDS after receiving 3 autologous activated PRP (aaPRP) treatments. All patients shared similar changes of leucocytes subsets with an increase leucocytes count but a reduced lymphocyte and monocytes count with an increase of neutrophils on the day of admission and during the observation periods. These findings were similar to haematologic findings in severe COVID-19 patients in Wuhan [6]. An increase of CRP was also found in these four patients as a signs of cytokine storm occurring.

A longitudinal study in Wuhan suggested that lymphophenia was due to a significant reduction of T cells, spesifically CD8+ T cells subset [6]. A reduction of this T cells subset was accompanied by an increase of inflammatory cytokines, like IL-2, IL-6, and IL-10 [6]. Manystudies have reported that chronic infectionor inflammation reduced the immune cells expansion and impaired the T cells function by manipulating the host's inflammatory cytokine milieu [16]. Another study also reported that high concentration of inflammatory cytokines induced the early death of erythroid progenitor cells in the bone marrow[17], thus hemoglobin level was frequently low in severe COVID-19 patients [18], like our patients. Hemoglobin is essential to carry oxygen to the body organs. Thus, low level of hemoglobin can lead to oxygen deprivation in multi-organs that may increase the mortality rate of COVID-19 patients.

As high inflammatory cytokines play significant roles in multi-organ failure that leads to death, it is very important and challenging to manage inflammatory outbursts in COVID-19 patients. Many treatments have been tested, including the use of steroid dexamethasone [19], anti-oxidant, such as NAC [20] and colchicine[21], even mesenchymal stem cells (MSCs) to control the inflammation [22]. Based on our experience in these four patients, the use of standard medication in combination with steroid, NAC, and/or

colchicine were not adequate to resolve the symptoms. Despite the benefit of MSCs in combating COVID-19 [23], as well as to induce tissue regeneration [23] that have been reported in several publications, we considered that the feasibility of MSCs was still limited due to the following reasons.

First, the safest MSCs for clinical application was autologous MSCs. However, the collection of MSCs sourced from COVID-19 patient's own body is not feasible. Even if it is possible to collect the MSCs source, the patient's clinical condition, such as diabetes mellitus [24] or inflammatory conditions, may negatively impact the ability of MSCs to proliferate during expansion period. This can result in a low number and low function of MSCs upon harvesting. In fact, the dosage of MSCs that was frequently used in many studies was 1 x 10^6MSCs per kilogram body weight or around 50−100 x 10^6MSCs per patient [23]. In general, this number of MSCs can be achieved at the minimum of 2 weeks, which can delay the treatment for COVID-19 patients.

Second, allogenic MSCs require healthy donors that would volunteer their body tissues, such as Wharton jelly's, bone marrow, or adipose tissue, as the source of MSCs. Donor screening and cells characterization should be done prior the use of allogenic MSCs to make sure that the cells are compatible with the recipients and not pose any virus or microbial organisms that may increase risk to benefit ratio of treatment. Similar to autologous MSCs, culturing the primary cells also needs 2 weeks at minimum. Current evidence suggested that MSCs are no longer immune-privileged [25]. The HLA-DR that play pivotal role in immune response pathway that should be diminished in MSCs can be re-expressed by the cells in certain conditions, such as inflammation [26]. These findings, therefore, make the potency and safety of MSCs treatment more limited.

Furthermore, it has been widely reported that regenerative and anti-inflammatory potency of MSCs was a result of its capability to regulate cells communication through various growth/trophic factors, chemokines, and cytokines, so called paracrine functions [27]. Some biological actives such as vascular endothelial growth factor (VEGF), transforming beta growth factor-1 (TGF-β1), insulin-like growth factor (IGF-1), platelet-derived growth factor (PDGF), and an anti-inflammatory IL-1RA secreted by MSCs were also contained in human platelets and can be released by simple ex-vivo platelet activation [28,29,30]. In inflammatory condition, adding PRP to the culture medium for nucleus pulposus-derived stem cells from early inflammed degenerated intervertebral discs may reduce several inflammatory genes expression, such as IL-1β, IL-6, IL-8, and TNFα and reduced IL-1β and TNFα inflammatory cytokines production as well [10]. So, instead of using MSCs, either autologous or allogenic, we used an aaPRP and explored its potency (efficacy and safety) in management of ARDS in severe COVID-19 patients. When combined with standard medication for COVID-19, aaPRP may increase the chance of patient to survive ARDS. We also observed that the sooner aaPRP was given to patient when ARDS onset, the lesser medications and aaPRP treatment were needed, thus reducing the hospital cost for COVID-19. Our experience in these four patients showed that the timing of aaPRP treatment is very important to achieve better outcomes. We considered that aaPRP treatment has a high chance to be safe and effective, both clinically and financially for COVID-19 patients.

On another note, platelets count in blood was high in these four patients that might occur as a compensatory mechanism to inflammatory conditions. The high platelet counts in blood may induce thrombosis in blood circulation, but it could offer some advantages in aaPRP preparations. PRP that are processed from blood with high platelet count will have a lot more platelet inside. Once it is activated in ex-vivo scenario, it may release more bioactive factors produced and packed within platelets to overcome the inflammation. This indicates that another research should be done to investigate whether aaPRP from COVID-19 patient with thrombocytosis has more benefits compared to COVID-19 patients with normal platelet counts or with thrombocytopenia.

In Indonesia, patients in the low to middle socioeconomic status use government health insurance to cover their medical costs. Thus, if aaPRP treatment is shown to be clinically safe and effective to accelerate healing and recovery process of COVID-19 patients, this will greatly reduce the economic burden on the government, as this treatment costs far less than MSCs treatment and requires a shorter hospitalization period.

4. Conclusion

Activated autologous PRP treatment could be a more feasible treatment for severe COVID-19 management compared to allogenic or autologous MSCs treatment and should be further investigated in a proper phase I and II clinical trial phase setting.

Consent participant

Treatment was reviewed and approved by medical board of Koja RPH and consent for treatment was given from every patients participated in this study.

Consent for publication

Patients were consent that their data will be used for publication with protection to their confidential information.

Availability of data and material

The datasets used and analysed during the current report are available from the corresponding author on reasonable request.

Authors' contribution

KK, LMC designed the scientific work. KK, LMC, IRI, and IRA reviewed the literature. KK, IRI, IRA wrote the first draft of the manuscript. LMC, NF, YH, and NP had responsibility of patient treatment and had complete access to the clinical and radiologic data of the reported cases. WRS, IA, TW, AZ, DE, NA hold responsibility for data input and tabulation. All the authors reviewed, revised, and approved the submitted manuscript.

Declarations of Competing Interest

The authors declare that have no conflict of interests.

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