# Convalescent Plasma Effect to Neutralization Antibody and Inflammation Parameter Level in Moderate COVID-19 Patients

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**Abstract.** The pandemic of COVID-19 continues with new variants. More than 401 million people in the world infected by COVID-19 and approximately 5.7 million people lost their lives. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has respiratory system as a target. This virus uses a receptor binding domain of SARS-CoV-2 Spike (sRBD) protein to bind ACE-2 receptor and enter human respiratory cells. The antibody to sRBD protein will block the virus. Convalescent plasma from recovered COVID-19 patient contains neutralization antibody which can neutralize sRBD and eliminate the virus. Therefore, this study aims to assess the therapeutic effect of convalescent plasma to neutralization antibody and inflammatory parameter level in moderate COVID-19 patients. This is a one-group pretest-posttest study of nine moderate COVID-19 patients admitted in Unggul Karsa Medika Hospital, Bandung, Indonesia. The study was done from August 1, 2021 to January 1, 2022. The level of neutralization antibody and inflammatory parameter were assessed before and after convalescent plasma therapy in moderate COVID-19 patient. Data analysis performed by SPSS program with $p < 0.05$ and dependent t-test. Our study shows that convalescent plasma therapy can influence the increase of neutralization antibody (142. 5 AU/mL vs 13.432 AU/mL, p value = 0.011) and the decrease of Interleukin-6 (IL-6) (41.61 pg/mL vs 14.5 pg/mL, p value = 0.008) and C-reactive protein (CRP) level (84.2 mg/L vs 17.4 mg/L, p-value = 0.018) in moderate COVID-19 patient. The patients were also getting better clinically, with the use of oxygen decreasing gradually from non-rebreathing masks to nasal cannula and from nasal cannula to room air after given convalescent plasma therapy. Therefore, we conclude that convalescent plasma therapy has a positive role in increasing neutralization antibody and decreasing inflammatory parameters which are IL-6 and CRP, thus had potential usefulness in therapy for moderate COVID-19 patients.

**Keywords:** COVID-19 · convalescent plasma · neutralization antibody · inflammatory parameters · Interleukin-6 · C-Reactive Protein


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## 1 Introduction

The pandemic of COVID-19 continues as the new variants emerges. More than 401 million people around the world infected by the virus and more than 5.7 millions lost their lives. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a virus which targeted respiratory system. In Indonesia, this virus contracts almost 4.6 million people and cause 144 thousand deaths until September 6, 2021. The virus spreads quickly and infect all ages from infant to senior person and people with comorbids such as diabetes mellitus, hypertension, and immunity disorders which could make their condition worse.

There is no definitive treatment to overcome the virus and one of the promising and useful therapy is convalescent plasma (CP). This passive immunity utilization dated back from Spain Flu between 1920 to 1925, Mumps, Measles, Ebola and SARS, involved convalescent plasma therapy (CPT), as in COVID-19 [5–12]. Many studies on CP have been and still being carried out with mixed result, but the study focus in neutralizing antibody and inflammatory parameter level is scarce especially in Indonesia. Recently, Indonesia has more than 4.2 millions COVID-19 survivors who has a huge potential to become CP donor [1].

In human cell, SARS-CoV-2 targets respiratory cells from upper respiratory tract to alveoli. Spike (S) protein Receptor Binding Domain (RBD) of SARS-CoV-2 will bind ACE-2 receptor before it can enter the cell, replicate it's genetic material and release new virions which are spread all over the body.

Virus antigen presentation will stimulate humoral and cellular immune response mediated by specific T and B cells. Humoral immune response will produce Immunoglobulin M (IgM) and Immunoglobulin G (IgG) to SARS-CoV-2, IgM will dissapear in the last periode of week 12 but IgG will have a stable level within four to six months and decrease gradually to undetectable level within two years. One of distinctive feature of SARS-CoV-2 is this virus can evade our immunity system through double membrane vesicle with no pattern recognition receptors (PRRs) and replicates within this vesicle so the host immune system failed to recognise and detect it.

Proinflammatory cytokine including TNF-α, IL-1, IL-6, IL-8 and infection marker as procalcitonine, ferritin and C-reactive protein will increase in the case of cytokine storm that usually occurs in severe and critical COVID-19. Cytokine storm result in Acute Respiratory Distress Syndrome (ARDS) which is the main cause of death in COVID-19 patient [23–28]. All of the damage originated from SARS-CoV-2, virus elimination is necessary before replication process and cytokine storm occurs. This is the place of CPT to reduce the virus amount and attenuate the rise of proinflammatory factors in cytokine storm.

## 2 Material and Methods

### 2.1 Subject

Subject of this study are moderate COVID-19 patients with positive Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) results based on standard COVID-19 patient diagnosis criteria in Indonesia. All patients were given and signed informed consent, agree to accept convalescent plasma therapy.

### 2.2 Methods

This is a one-group pretest-posttest study and started from August 2021 to January 2022, consisted of nine moderate COVID-19 patients from total twelve patients who had agreed to accept CPT and given consent to be included in this study with consecutive sampling. All patient have at least one comorbid condition and fulfilled the moderate criteria of COVID-19 including cough, fever and dyspnoe, respiratory rate more than 20 times/minutes and saturation less than 92% in room air.[29, 30].

Each patient's blood was drawn twice, within one hour before and four hours after convalescent plasma was given. Neutralizing antibody and proinflammatory parameter level examination would be performed on patient serum from the blood sample. Every patient received 500–800 cc CP, divided in two to four bags of CP, administered every 48 h, each bag was given within four hours. All CP provided by Indonesia Red Cross wih antibody threshold limit above 1:320.

Neutralization antibody and Ferritin were measured by Abbott Architect, Interleukin-6 was measure by Cobas E601 and DDimer was measured by Vidas, all of these measurement were done by Prodia. C-Reactive Protein, Procalcitonin and Troponin I were measured by Wondfo Finecare FIA Meter. Ureum, Creatinin, SGOT, SGPT, Albumin, Globulin, Total Protein and LDH were measured by Cobas C111. All of these measurement were done at Unggul Karsa Medika Hospital Laboratory.

### 2.3 Statistic

There are two sets of data, neutralizing antibody and proinflammatory parameter level one hour before and four hours after CPT in moderate COVID-19 patient. Data analysis performed by SPSS program with significancy $p < 0.05$ with normality test and t-test.

## 3 Results

### 3.1 Patient Characteristics

There are nine moderate COVID-19 patients ($n = 9$) in this study, including five males and four females. Mean age was 57.5 years (range 26 to 83 years). Most of the symptoms were fever, non-productive cough, dyspnoe and diarrhoea. Eight patients received CP within 2 to 3 days after hospital admission and one patient received CP on day 20. Mean inpatient days was 11.5 day (range 5 to 25 days).

### 3.2 Respiration Parameter Before and After Convalescent Plasma Therapy

All patients showed respiratory parameters improvement after CPT including respiration rate, oxygen support and saturation. Before CPT, all patients used oxygen support from nasal cannula (NC) to high flow nasal cannula (HFNC). After CPT, six patients were no longer using oxygen support, one patient switched from non-rebreathing mask (NRM) to NC, two patients used NC and NRM with lower oxygen flow.

**Table 1.** Patient characteristic.

| Patient number | Gender | Age (yo) | Clinical Stage | Hospital Admission to CPT | Total Inpatient Days | Comorbidity Number | Comorbidity |
|---|---|---|---|---|---|---|---|
| 1 | Female | 26 | Moderate | 2 | 5 | 1 | Asthma |
| 2 | Male | 72 | Moderate | 20 | 25 | 2 | Hypertension, Diabetes Mellitus |
| 3 | Female | 65 | Moderate | 2 | 9 | 2 | Diabetes Mellitus, Hypertensive Heart Disease |
| 4 | Male | 35 | Moderate | 2 | 7 | 2 | Diabetes Mellitus, Hypertension |
| 5 | Female | 83 | Moderate | 2 | 7 | 3 | Atrial Fibrillation, Congestive Heart Failure |
| 6 | Male | 51 | Moderate | 2 | 13 | 3 | Diabetes Mellitus, Hypertension |
| 7 | Female | 54 | Moderate | 3 | 10 | 4 | Hypertension, Hypertensive Heart Disease, Coronary Artery Disease, Suspected TBC |
| 8 | Male | 57 | Moderate | 2 | 10 | 1 | Hypertensive Heart Disease |
| 9 | Male | 75 | Moderate | 2 | 9 | 1 | Diabetes Mellitus |

Abbreviations: yo: years old; CPT: convalescent plasma therapy

### 3.3 Laboratory Results

There was a significant increase of neutralizing antibody level with $p = 0.011$ ($p < 0.05$) and a significant decrease of Interleukin-6 with $p = 0.008$ ($p < 0.05$) and CRP with $p = 0.018$ ($p < 0.05$) after convalescent plasma therapy.

**Table 2.** Respiration parameter before and after convalescent plasma therapy.

| Patient | Respiration | | Oxygen Support | | Oxygen Saturation | |
|---|---|---|---|---|---|---|
| Number | Before CPT | After CPT | Before CPT | After CPT | Before CPT | After CPT |
| 1 | 20 | 20 | NC 5 L/m | Room air | 96 | 98 |
| 2 | 22 | 21 | NC 5 L/m | NC 3 L/m | 98 | 98 |
| 3 | 27 | 18 | NRM 15 L/m | NC 5 L/m | 94 | 99 |
| 4 | 30 | 20 | NRM 10 L/m | Room air | 98 | 97 |
| 5 | 15 | 13 | NC 3 L/m | Room air | 98 | 100 |
| 6 | 29 | 20 | HFNC 40 L/m | Room air | 99 | 97 |
| 7 | 29 | 23 | NRM 15 L/m | NRM 13 L/m | 98 | 99 |
| 8 | 24 | 19 | NC 6 L/m | Room air | 97 | 97 |
| 9 | 23 | 16 | NC 2 L/m | Room air | 97 | 99 |

**Abbreviations:** CPT: convalescent plasma therapy; NC: nasal canule; NRM: non-rebreathing mask; HFNC: high-flow nasal canule; L/m: litre per minute

**Table 3.** Comparison of neutralizing antibody and proinflammatory parameter level before and after convalescent plasma therapy.

| Laboratorium Parameter | Before CPT (n = 9) | After CPT (n = 9) | p-value |
|---|---|---|---|
| Neutralizing Antibody (AU/mL) | 142.5 (50 – 18,036.7) | 13,432 (1,056 – 40,000) | .011 |
| Ferritin (ng/mL) | 1,195.2 (159.9 – 3,382.1) | 915.65 (132.4 – 1,080.2) | .349 |
| DDimer (mg/dL) | 1 (0.1 – 6.5) | 0.4 (0.1 – 4.8) | .663 |
| Interleukin-6 (pg/mL) | 41.61 (8.04 – 70.4) | 14.5 (3.48 – 37.25) | .008 |
| CRP (mg/L) | 84.2 (9.4 - 200) | 17.4 (2.7 – 71.3) | .018 |
| Procalcitonin (ng/mL) | 0.48 (0.21 – 4.5) | 0.2 (0.1 – 2.3) | .121 |
| SGOT (IU/L) | 29 (25 - 91) | 23 (11 - 49) | .532 |
| SGPT (IU/L) | 23 (11 - 81) | 18 (11 - 62) | .701 |
| LDH (U/L) | 341 (87 - 525) | 185 (75 - 325) | .069 |
| Ureum (mg/dL) | 34 (12 - 82) | 32 (19 - 71) | .961 |

(*continued*)

**Table 3.** (*continued*)

| Laboratorium Parameter | Before CPT (n = 9) | After CPT (n = 9) | p-value |
|---|---|---|---|
| Creatinin (mg/dL) | 0.9 (0.6 – 1.3) | 0.8 (0.3 – 1.1) | .133 |
| Troponin I (ng/mL) | 0.2 (0.1 – 1.3) | 0.1 (0.1) | .04 |
| Total Protein (g/dL) | 6.9 (5.5 – 8.3) | 6.9 (5.4 – 7.3) | .823 |
| Albumin (g/dL) | 3.8 (2.9 – 4.4) | 3.5 (3 – 4.5) | .405 |
| Globulin (g/dL) | 2.8 (2 – 3.9) | 3.3 (1.8 - 4.5) | .764 |

**Abbreviations**: CPT: convalescent plasma therapy; AU: arbitrary unit; mL: mililitre; ng: nanograms; pg : picograms; mg: miligrams; IU: international unit; g: grams; L: litre; U: unit; dL: decilitre

## 4 Discussion

In this study, all patients were given 500 to 800 cc of convalescent plasma (CP) divided in two to four doses. The mean of neutralizing antibody level before CPT was 142.5 AU/mL and after CPT was 13.432 AU/mL, showed a significant increase with $p = 0.011$ ($p < 0.05$). All patients showed negative RT-PCR result after CPT and this is in accordance with study by Franchini. This result also shows the possibility of convalescent plasma protective effect for people with certain conditions who can not receive the vaccine [30–35].

C-Reactive Protein (CRP), as progressivity marker, decreased one day after CPT. This study also found a significant decrease of CRP from 84.2 to 17.4 with $p = 0.018$ ($p < 0.05$) after CPT. A cohort study by Salazar and colleagues in 25 severe COVID-19 patients, a significant decrease of CRP were shown at day 7 and 14 after CPT from 14.66 mg/dL before convalescent plasma transfusion to 2.9 mg/dL at day 7 and 0.45 mg/dL at day 14 after convalescent plasma transfusion [5].

We also observed a high pro-inflammatory cytokine IL-6 level in some patients before convalescent plasma transfusion. The increase of IL-6 level is an indicator of poor outcome in severe COVID-19 patients with pneumonia and acute respiratory disease syndrome as a result of cytokine storm (35). This study showed a significant decreasement of IL-6 with $p = 0.008$ ($p < 0.05$). Early convalescent plasma treatment can decrease endogen inflammatory process and relieve immunity system besides being able to increase neutralizing antibody level [35–39].

### Limitation

This is the first one-group pretest-posttest study of convalescent plasma therapy in Indonesia to examine the effect of convalescent plasma in moderate COVID-19 patient and the result is very promising. Even though, this study has some limitations. First, the study performed on only nine patients. Besides the very promising result, larger patients number and randomized control trial (RCT) are needed in order to increase validity of the study, despite difficulties to perform an RCT in COVID-19 patient both in severe

and critical stages. Second, patient also received other antiviral drugs besides convalescent plasma. Despite the uncertainty efficacy of the drugs, there is the possibility of a relation between convalescent plasma and drugs. Further study is needed to differentiate the effect of convalescent plasma and antiviral used.

## 5 Conclusions

Convalescent plasma has a positive effect in increasing neutralizing antibody level and reducing CRP and IL-6 in moderate COVID-19 patient. In COVID-19, CRP and IL-6 are inflammatory cytokine and will reach a high level during cytokine storm, causing tissue damage and death. This exaggerated inflammatory effect will be more severe in comorbid patient because the immune system fails to function optimally in eliminating the virus and producing antibody. There are several factors influence the successful of this treatment including the level of neutralizing antibody in the convalescent plasma, the time convalescent plasma was given and the convalescent plasma quantity needed based on patient comorbidity condition. Early convalescent plasma therapy can prevent or relieve the cytokine storm which have a devastating effects especially on comorbid patients.

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