The Role of Inflammatory Parameters and Antibody Seroconversion on COVID-19 Outcomes in Patients with Central Obesity

Syahidatul Wafa[1,2], Dicky Levenus Tahapary[1,2], Evy Yunihastuti[3], Heri Wibowo[4,8], Cleopas Martin Rumende[5], Kuntjoro Harimurti[6], Ketut Suastika[7], Farid Kurniawan[1,2], Rona Kartika[2], Tika Pradnjaparamita[2], Dante Saksono Harbuwono[1,2]*

[1]Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.
[2]Metabolic Disorder, Cardiovascular and Aging Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia.
[3]Division of Allergy and Immunology, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.
[4]Integrated Laboratory, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.
[5]Division of Respirology and Critical Care Department of Internal Medicine, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.
[6]Division of Geriatric, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia.
[7]Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Universitas Udayana, Denpasar, Bali, Indonesia.
[8]Department of Parasitology, Faculty of Medicine, Universitas Indonesia Hospital, Jakarta, Indonesia.

Corresponding Author:
Dicky Levenus Tahapary, MD., PhD. Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine, Faculty of Medicine Universitas Indonesia-Dr. Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta 10430, Indonesia. Email: dicky.tahapary@ui.ac.id.

ABSTRACT

***Background**: Central obesity increases the risk of developing poor outcomes of COVID-19. The pro-inflammatory state and antibody dysfunction are thought to contribute to poor outcomes; however, the evidence is unclear.* ***Methods**: This is a cohort study among COVID-19 patients with central obesity in Dr. Cipto Mangunkusumo National General Hospital Jakarta, Indonesia, during the early phase of the COVID-19 pandemic. Our study is a part of the COVID-19, Aging, and Cardiometabolic Risk Factors (CARAMEL) study. From the CARAMEL study, we selected adult non-ICU/HCU inpatient subjects with central obesity that met inclusion/exclusion criteria, collected clinical and anthropometric data, and measured inflammatory cytokines and IgG S-RBD SARS-CoV-2 antibody titers from a stored sample taken at day 2 of hospitalization . The poor clinical outcome of hospitalization was observed. We used the Mann-Whitney test to analyse non-normally distributed data, and T-test for normally-distributed data. The adjusted-relative risk of negative seroconversion antibody for poor outcomes was analysed using logistic regression.* ***Results**: 23 of 178 (12.9%) subjects developed poor clinical outcomes during hospitalization. Subjects with poor outcomes had a higher visceral fat area (14.5 vs. 11 , $p < 0.05$), waist circumference and BMI. The level of CRP, pro-inflammatory cytokines (IL-6 and MCP-*

1) and anti-inflammatory cytokines (IL-1Ra, IL-4, and IL-10) were significantly higher in subjects with poor outcomes, alongside with the lower antibody titer in subjects with poor outcomes. Antibody seroconversion failure increased the risk of developing poor outcomes (aRR 2.696, 95% CI 1.024-7.101), after adjusting for age and sex. ***Conclusion****: In COVID-19 patients with central obesity, we confirmed the association between higher pro- and anti-inflammatory parameters, and lower SARS-CoV-2 antibody with poor outcomes of COVID-19.*

Keywo*rds: inflammatory parameters, antibody response, COVID-19 severity, central obesity, poor outcome.*

INTRODUCTION

Obesity, particularly central obesity, is a well-established risk factor for the development of cardiovascular and metabolic disease.[1] Besides cardiometabolic disease, obesity is also related to the severity of infectious disease. During the COVID-19 pandemic, obesity has been proven as an independent prognostic factor of poor outcomes, such as mortality, intensive care unit (ICU) and mechanical ventilations requirements.[2] While visceral fat predicts obesity complications better than body mass index (BMI) measurement,[3,4] the study about clinical outcome of infections in central obesity is still limited. Previous meta-analysis[5] reported that visceral fat area is higher in COVID-19 patients with ICU/ventilator requirements and suggested that subcutaneous fat area does not predict critical conditions. The pro-inflammatory state and defective immunity due to excess visceral fat are thought to are thought be contributing factors of COVID-19 poor outcomes in central obesity; however, the evidence is still unclear.

The pro-inflammatory states in central obesity is caused by a higher production of proinflammatory cytokines in visceral adipose tissue. The hypertrophy/hyperplasia of visceral adipose tissue leads to local inflammatory conditions through adipocyte hypoxia and necrosis that recruits many inflammatory cells into adipose tissue. The recruited macrophages would change into proinflammatory macrophages (M1) that secrete many proinflammatory cytokines, and anti-inflammatory macrophages (M2) phenotype would be suppressed. The high production of proinflammatory cytokines from adipose tissue would spill into circulation and lead to a systemic inflammatory state.[6-8]

In the other hand, the chronic inflammatory state in obesity might play a role in suppressing antibody response to infection or vaccination, leading to defective immunity. The suboptimal immune response in obesity renders certain phenomena of antibody response similar to aging, called immunosenescence in obesity.[9] Previous study [10] reported the negative association between BMI and SARS-CoV-2 antibody response in COVID-19 patients with obesity. Besides antibody titers, the time of antibody development was considered essential to optimal defense from infections. The delayed kinetics of antibody response in COVID-19 is associated with poor outcomes.[11, 12] Since the development of SARS-CoV-2 antibody has been observed during the first 14 days of COVID-19 infections,[13] it is hypothesized that antibody seroconversion failure during the first 14 days of symptom onset might contribute to poor viral clearance of SARS-CoV-2, allowing the virus to propagate the infection through multiple organs and resulting in severe conditions.

The relevance of inflammatory state and defective immunity, as assessed by antibody response, in central obesity is well known. However, the role of inflammatory parameters and antibody response to the development of poor infection outcomes in central obesity is less well characterized. Therefore, given the rising obesity rates and the renewed circulation of respiratory viruses, it is crucial to understand how the inflammatory parameters and antibody response in central obesity influence the risk of poor outcomes. This study aims to determine the relationship between inflammatory parameters and antibody response during the first 14 days of symptom onset with poor outcomes in hospitalized COVID-19 patients.

METHODS

Study Design and Ethics

We performed a cohort study among newly diagnosed adult COVID-19 patients (confirmed with RT-PCR) in Dr. Cipto Mangunkusumo National General Hospital in Jakarta, Indonesia during early phase of COVID-19 pandemic (December 2020 to March 2021). Our study is a part of the CARAMEL (COVID-19, Aging, and Cardiometabolic Risk Factors) study.[14] From the baseline data of the CARAMEL study, we selected only the hospitalized patients in general wards (non-ICU/high care unit [HCU]) that met the following inclusion criteria: (1) symptom onset duration was within 0–14 days when CARAMEL team drew the blood; (2) aged 18–65 years old; (3) clinical severity was mild or moderate when admitted to hospital; (4) had no poor outcome before recruitment; (5) no history of COVID-19 vaccination; (6) had data on waist circumference. Based on waist circumference data, we excluded subjects without central obesity. We followed up all subjects until they had poor outcomes or were discharged—a centralized database stores follow-up data for patient outcomes during hospitalization. This study has been approved by the Ethical Committee Board Faculty of Medicine Universitas Indonesia (KET-1112/UN2.F1/ETIK/PPM.00.02/2020).

Demographic and Clinical Data

This study took demographic and clinical data from the CARAMEL study, including age, gender, COVID-19 symptoms, onset of symptoms, comorbidities and smoking history.

Anthropometric Measurement

In CARAMEL study, the anthropometric measurements were performed on patients without poor clinical conditions. Participants' waist circumference was measured using a measuring tape (SECA Model 201, Seca GmbH Co, Hamburg, Germany) taken on patients standing position during normal breathing. The measurement location is in the abdomen at the midpoint between the lowest rib and the endpoint of the groin. Body weight, fat percentage, and visceral fat were determined using a mobile flat scale with Bioelectric Impedance Analysis (Tanita Model BC-601, Tanita Corp, Tokyo, Japan). Body height was measured using a portable stadiometer (SECA Model 206, Seca GmbH Co, Hamburg, Germany). Using body weight and height, BMI was calculated. Based on WHO Asia Pacific Criteria, obesity was diagnosed as a BMI ≥ 25 kg/m^2, overweight as a BMI 23–24.9 kg/m^2, and central obesity as a waist circumference ≥ 80 cm in females and ≥ 90 cm in males. [15]

Inflammatory Parameters

The inflammatory cytokines (IL-6, TNF-α, IL-1RA, IL-1 β, MCP-1, IL-10, dan IL-4) were measured from the stored serum using bead-based multiplex assays methods and read in median fluorescent intensity (MFI) using LUMINEX 200 (See Supplementary). C-reactive protein was measured using ECLIA methods (Abbott, Abbott Park, Illinois, USA).

Serum SARS-CoV-2 Antibody Measurement

Quantitative SARS-CoV-2 IgG antibody titer was measured using the Abbott chemiluminescent microparticle immunoassay (CMIA). This assay can measure antibody titer in the range of 21–40,000 AU/mL. The titer below 50 AU/mL was concluded as an antibody seroconversion failure, while titer at or more than 50 AU/mL would concluded as no antibody seroconversion failure.

Assessment of Poor Clinical Outcomes

Poor clinical outcomes were defined by the presence of any of the following conditions: worsening of COVID-19 severity from mild or moderate to severe, intensive care unit admission requirements, acute respiratory distress syndrome (ARDS), and death during hospitalization. ARDS was defined using Kigali's modification of the Berlin criteria.[19]

Data Analysis

The value of α was set at 5%, while the statistical power was 90%. Normality data distribution was tested using the Kolmogorov-Smirnov test. Mean and standard deviation were used to describe data in a normal distribution, while the median was used to describe data in a non-normal distribution. The mean difference between the two groups was compared with an independent t-test for normally distribute data and a Mann-Whitney test for non-normally distributed data. The relationship between

two categorical variables would be analysed a Chi-square test and the multivariate analysis would be conducted using a Logistic regression. Statistical analysis was performed using SPSS version 20.0. Statistical significance was set at a p-value of < 0.05. The graph was drawn using GraphPad Prism.

RESULTS

Study Population

From 440 CARAMEL study subjects, there were 396 patients hospitalized in general wards; of those subjects, 178 with central obesity were selected for this study (**Figure 1**). We revealed that 23 subjects (12.9%) developed poor outcomes during follow-up. The poor outcomes were mortality (3 subjects [1.7%]), ARDS and requirement of ICU care (17 subjects [9.6%]), and worsening oxygen requirements without developing ARDS/ ICU requirement (4 subjects [2.2%]). All of the deceased patients had ARDS before death. Subjects developing poor outcomes had a significantly higher CRP, visceral fat, and a trend of higher BMI and waist circumference compared to subjects without poor outcomes. (**Table 1**)

Table 1. Baseline characteristics of subjects

	Total N = 178	Poor outcomes		P-value (*)
		Yes (N = 23)	No (N = 155)	
Demographic and Clinical Data				
Age, years, median (IQR)	46.5 (33–55)	51 (32-57)	46 (33-55)	0.32
Female sex, n/N	90/178	10/23	80/155	0.47
Clinical severity at hospital admission				0.29
Mild, n/N	82/178	8/23	74/155	
Moderate, n/N	96/178	15/23	81/155	
Comorbidities				
Diabetes n, %	86 (45.7)	13 (50)	73 (45.1)	0.64
Hypertension n, %	56 (29.8)	7 (26.9)	49 (30.2)	0.73
Dyslipidemia n, %	45 (23.9)	6 (23.1)	39 (24.1)	0.91
Chronic kidney disease, n, %	9 (4.8)	2 (7.7)	7 (4.3)	0.46
Cardiac disease, n, %	12 (6.4)	1 (3.8)	11 (6.8)	0.57
COPD, n, %	3 (1.6)	0 (0)	3 (1.9)	0.49
Liver disease, n, %	1 (0.5)	0 (0)	1 (0.6)	0.69
Smoking, n, %	54 (28.7)	10 (38.5)	44 (27.2)	0.24
Onset of symptom onset, days, mean (SD)	6 (3.1)	5.6 (2.7)	6.7 (3.2)	0.25
Anthropometric Data				
BMI, kg/m^2, median (IQR)	28.5 (27.8–29.2)	30 (26.2–35.4)	28 (25.3–31.4)	0.07
Waist circumference, cm, median (IQR)	92.5 (86.8–100)	102 (93–112)	97 (90–103)	0.06
Male	102 (100–104)	105 (96–118)	100 (94–107)	0.11
Female	94 (92–96)	97 (90–105)	92 (87–100)	0.25
Visceral fat area, median (IQR)	11 (8-15)	14.5 (10-17)	11 (8-15)	0.041
Body fat percentages, %, mean (SD)	35 (8.5)	34.8 (10.4)	35.1 (8.3)	0.88
Laboratory Data				
CRP, mg/L, median (IQR)	14.5 (5.4–51)	22 (14–57.5)	10.6 (3.9–40.5)	0.002
Mild infection	9.8 (3.5–23.4)	18 (5.7–54.7)	8.7 (3.5–23.4)	0.27
Moderate infection	17.3 (5.6–57)	37.6 (17–57.5)	15 (5.3–56.7)	0.047

*) p-value of comparison between subjects with poor outcomes compared to those without poor outcomes. P-value < 0.05 means statistically significant.

Inflammatory Cytokines of Subjects with vs. without Poor Outcomes

In terms of proinflammatory cytokines, we observed the higher IL-6 and MCP-1 levels in subjects with poor outcomes than without poor outcomes, which are 4.2 pg/mL (IQR 2.5–10.1) vs. 2.1 pg/mL (IQR 1.7–2.4), $p < 0.01$ for IL-6 (Fig. 1A) and 479 pg/mL (IQR 290–643) vs. 205 pg/mL (IQR 130–368), $p < 0.001$ for MCP-1 (Fig. 1B). Similar trend was seen in the level of TNF-α and IL-1β (**Figure 1C** and **1D**).

We also observed higher anti-inflammatory cytokines (IL-1Ra, IL-10 and IL-4) levels in subjects with poor outcomes compared to without poor outcomes. The most significant difference was seen in IL-1Ra levels, which are 1,896 (IQR 1,238–5,859) vs. 668 (363–1,446), $p < 0.001$ in poor outcomes vs. no poor outcomes, respectively (**Figure 1F**). The further analysis shown that the associations between IL-6, MCP-1, IL-1Ra, IL-10 and IL-4 with poor outcomes were seen both in subjects came to hospital with mild infections, and with moderate infections (**Table 1**)

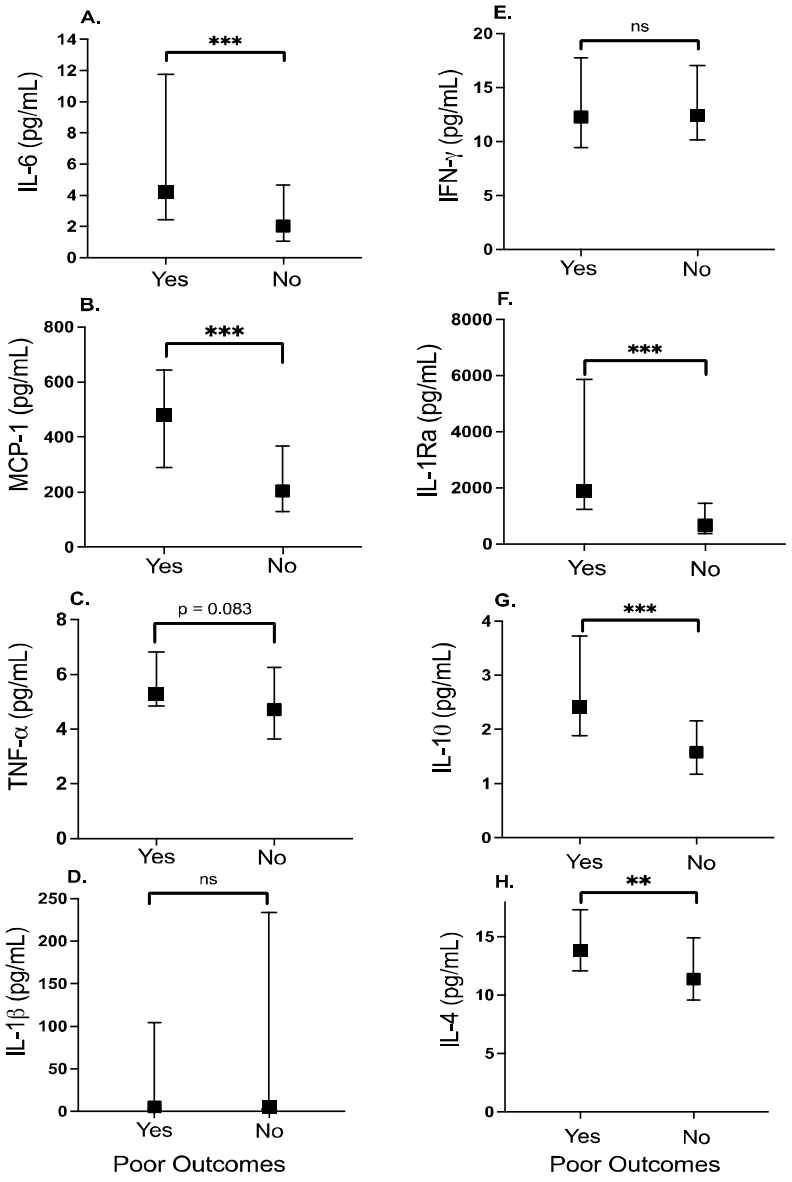

Figure 1. Association between pro- and anti-inflammatory cytokines with poor outcomes in COVID-19 patients with central obesity. The cytokines levels: IL-6 (A), MCP-1 (B), TNF-α (C), IL-1β (D), IFN-γ (E), IL-1Ra (F), IL-10 (G) and IL-4 (H), are presented as median (IQR).
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Antibody Response in Subjects with vs. without Poor Outcome

Our study observed that antibody seroconversion failure during the first 14 days of symptom onset increases the risk of poor outcomes after adjusting to age and sex, whether in total subjects (**Figure 2A**), or in subjects admitted to hospital with moderate infection (**Figure 2B**). However, in subjects with mild infection, there was no significant result (**Figure 2C**). There was also a lower IgG S-RBD SARS-CoV-2 antibody titer among subjects with poor outcome particularly among patients admitted to the hospital with moderate infection, but no significant result in mild infections (**Figure 2**).

DISCUSSION

This study demonstrated the association between higher pro-inflammatory parameters (CRP, IL-6, MCP-1, TNF-α) and anti-inflammatory parameters (IL-1Ra, IL-10 and IL-4) with poor clinical outcomes of COVID-19 in patients with central obesity. We also observed the higher risk of poor outcomes in subjects with antibody seroconversion failure.

A chronic proinflammatory environment with increased plasma levels of several inflammatory parameters is well established as a hallmarks of obesity.[7, 16] Therefore, several studies correlated the clinical outcomes of infections and the cytokines profiles to assess if high levels of these inflammatory cytokines influences the clinical outcomes of infection in individuals with obesity, [17-19] however, the result is inconsistent, and the study in central obesity is still limited.

Our study supported previous studies that reported the role of IL-6 and MCP-1 cytokines to the severity of COVID-19 infections [19-21] The higher initial IL-6, TNF-α and IL-1 levels were documented in non-surviving patients that were hospitalized in ICU with ARDS.[22] The baseline higher pro-inflammatory state in central obesity would be exaggerated when individuals were exposed to antigens from the virus, leading to a cytokine storm and poor clinical outcomes. Upon virus invasion of epithelial cells, neutrophils and macrophages were activated by recognizing pathogen-associated molecular patterns (PAMP) in the viral body. [23] Defending against the virus, M1-macrophages secretes several pro-inflammatory cytokines/chemokines such as IL-6, TNF-α, IL-1 and MCP-1. IL-6, being the foremost cytokines in the acute phase of inflammation. Through the JAK/STAT pathway and MAPK cascade, IL-6 plays an important role in activating other acute-phase proteins such as CRP, stimulation of complement response to opsonize pathogens and improving acquired immunity against pathogens. [18, 21, 24, 25] TNF-α stimulates lipolysis and release of free fatty acids that worsen proinflammatory state. [12, 26] MCP-1, a chemotactic cytokine (chemokine), recruits more monocytes into the infection site. MCP-1 directs the movement and infiltration of monocytes into the infection site through MAPK signalling pathways, and upregulates the production of ROS in site of inflammation.[27]

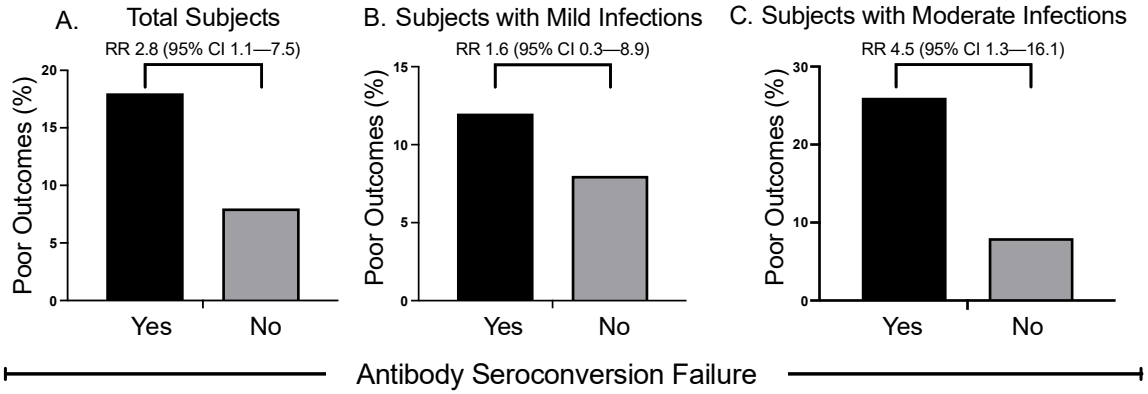

Figure 2. The relationship between IgG S-RBD SARS-CoV-2 antibody seroconversion failure and poor outcomes in total subjects (A), subjects admitted to hospital with moderate infections (B) and mild infections (C) was analyzed with logistic regression with adjustment of age and sex.

Besides the pro-inflammatory cytokines, our study also observed the association between higher level of anti-inflammatory cytokines (IL-1Ra, IL-10 and IL-4) and poor outcomes. This result supported previous study results from Zhao et al. [28] that observed the associations between IL-10 and IL-1Ra with disease severity in COVID-19 patients with obesity. The increase in anti-inflammatory cytokines is a compensatory response to the increase in pro-inflammatory cytokines that aims to limit the excessive and prolonged pro-inflammatory response as a counterpart regulation mechanism. This mechanism reflects an efficient autocrine mechanism in controlling the production of cytokines from monocytes/macrophages to resolve inflammation, prevent immunopathology and maintain cellular homeostasis. [29-31] Therefore, after exposure to LPS, there is a quick increase of IL-6 ,TNF-α and IL-1β, followed by a gradual rise in IL-10. The increased IL-10 aims to control IFN-γ production from Th1, resulting in a switching transition from IFN-γ to IL-10. The same phenomenon was found in IL-1Ra response to elevated IL-1β. A higher IL-1Ra concentration (100 times higher) is required to combat IL-1β elevation. Along with activation of the adaptive immune response, innate immune response would be entirely resolved.[29,30] However, the elevation of anti-inflammatory cytokines could not protect the subject from progression of infection due to unabated excessive inflammation in hyper-inflammatory response and leads to cytokine storm.

Our study observed that SARS-CoV-2 IgG S-RBD antibody seroconversion failure at the first 14 days of symptom onset increases the risk of poor clinical outcomes of COVID-19 patients with central obesity. Furthermore, the level of IgG S-RBD SARS-CoV-2 titer was lower in subjects with poor outcomes than without poor outcomes. This result supports previous studies. Takita et al. [32] reported that the SARS-CoV-2 IgG antibody titer on day 14 of symptom onset was lower in non-survivor patients (0.01 AU/mL) than in survivors (0.42 AU/mL), p = 0.02). Ren et al.[11] reported that slower antibody development correlated with more severe manifestations of COVID-19. Similar to Ren, Lynch et al.[33] showed that inpatient COVID-19 patients who required intensive care showed delayed development of peak antibodies compared to patients who did not require intensive care. The formation of adequate antibody levels at the first 14 days of symptom onset is essential to prevent poor clinical outcomes. This is related to the role of an adequate antibody response in the first 14 days of symptom onset in neutralizing the virus and preventing subsequent viral activity. The unabated virus replications leads to cytokine storm and poor clinical outcomes.

To the best of our knowledge, our study is the first study that documented the relationship between pro-inflammatory state and antibody seroconversion failure with poor clinical outcomes of COVID-19 in individuals with central obesity. However, the assessment of symptom onset was conducted based on history taking that may lead to the potential risk of bias. However, this risk was considered low because CARAMEL study was conducted during the first phase of COVID-19 infections that any individuals with COVID-19 symptoms would delicately noticed the onset of symptom and aggressively seek of health care to prevent worse conditions.

CONCLUSION

Higher pro- and anti-inflammatory parameters and lower antibody response were associated with poor infection outcomes in COVID-19 patients with central obesity.

Future Directions

Central obesity is related to inflammatory profile changes and disruption of immune response. Further studies are needed to elaborate the role of pro-inflammatory state in antibody disruption, and to analyse the relationship between inflammation and immunological response in central obesity.

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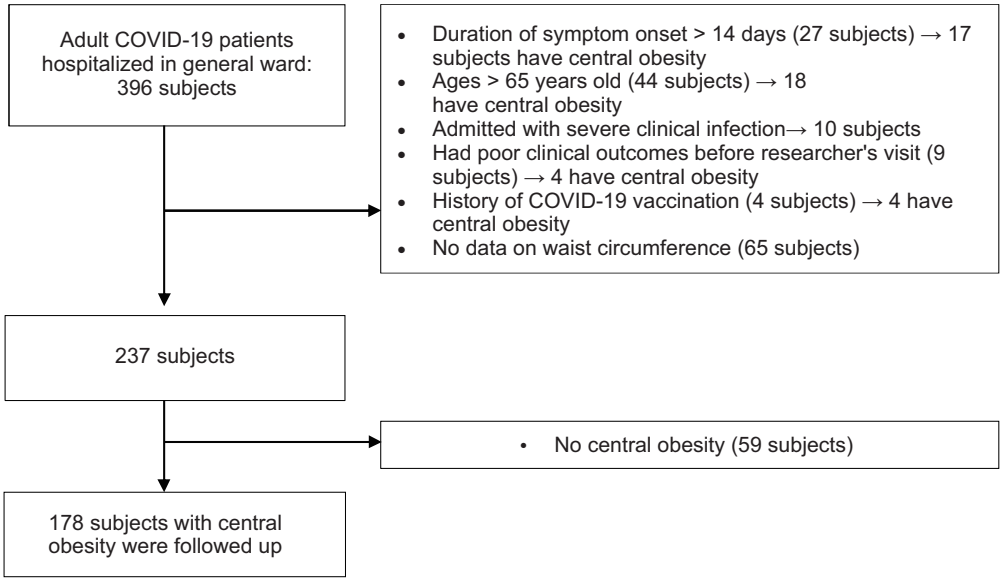
SUPPLEMENTARY

Measurement of Inflammatory cytokines

The inflammatory cytokines (IL-6, TNF-α, IL-1RA, IL-1 β, MCP-1, IL-10, dan IL-4) were measured using bead-based multiplex assays. As much as 25 mL serum from sample and standard was inserted into the 96-wells plate and mixed with 25 L mixed magnetic beads. Then, plate was incubated and agitated for 2 hrs in room temperature, and washed using 200 mL wash buffer 3x. After that, antibody was added into each well and incubated for 1 hr. Then, 25 mL streptavidin phycoerythrin was added into each well and incubated again for 30 minutes. After that, plate was washed and sheath fluid was added. The sample in plate was read in median fluorescent intensity (MFI) using LUMINEX 200.



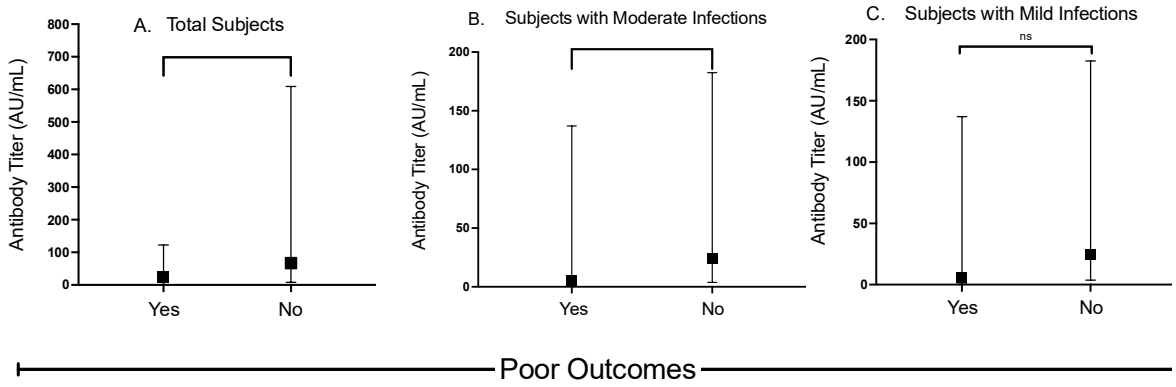
Supplementary Figure 1. Subject Recruitment Process

Supplementary Table 1. The associations of cytokines with poor outcomes during hospitalization according to severity of infection at hospital admission.

	Poor outcomes	Non-poor outcomes	P-value *)
Pro-inflammatory cytokines			
IL-6, pg/mL, median(IQR)			
Mild infection	3.2 (1.8−39)	1.9 (1.2−3.9)	0.085
Moderate infection	3.2 (2.9−11.5)	2.3 (0.9−6.3)	0.008
MCP-1, pg/mL, median(IQR)			
Mild infection	582 (361−772)	209 (140−428)	0.005
Moderate infection	351 (263−642)	198 (109−325)	0.001
Anti-inflammatory cytokines			
IL-1Ra, pg/mL, median(IQR)			
Mild infection	2,140 (1,288−1419)	660 (237−1,542)	0.005
Moderate infection	1,896 (771−3,203)	684 (417−1,215)	0.006

IL-10, pg/mL, median(IQR)			
Mild infection	2.7 (2.1−10.9)	1.6 (1.2−2.2)	0.014
Moderate infection	2.3 (1.8−3.7)	1.5 (1.1−2.1)	0.001
IL-4, pg/mL, median(IQR)			
Mild infection	14.3 (12.5−19.8)	12.1 (9.3−17.8)	0.102
Moderate infection	13.7 (10.6−15.5)	11.3 (9.6−14.2)	0.05

*) Analyzed using Mann-Whitney Test. P-value < 0.05 is considered statistically significant.



Supplementary Figure 2. The relationship between IgG S-RBD SARS-CoV-2 antibody titer and poor outcomes in total subjects (A), subjects admitted to hospital with moderate infections (B) and mild infections (C) was analyzed with Mann-Whitney test. The titer of antibody was presented as median (IQR) values.