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Original Article

Specific dysregulation of inflammatory and anti-inflammatory cytokine responses in pregnant women with COVID-19 infections

[Desregulación específica de las respuestas de citocinas inflamatorias y anti-inflamatorias en mujeres embarazadas con infecciones por COVID-19]

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Abstract

Context: Owing to its extensive inflammation, COVID-19 infection may have a specific response in the anti-inflammatory milieu of pregnancy.

Aims: To analyze the lacking evidence of systemic inflammatory and anti-inflammatory cytokine responses of pregnant women with COVID-19 infection compared to normal pregnancies.

Methods: This case-control study was performed on third-trimester symptomatic COVID-19 pregnant women compared with normal pregnancies without other significant inflammation risks. A between-group analysis was conducted to assess the inflammatory, and anti-inflammatory cytokines and chemokines. Through a comparison and correlation analysis, we detected differences between cytokines in the COVID-19 group based on the severity of the infection.

Results: Pregnant women with COVID-19 had higher procalcitonin levels ($p<0.01$), IL-6 ($p=0.05$), TNF-α, IFN-γ/IL-4 ratio ($p<0.01$), and lower IL-4 ($p<0.05$), implying a higher proinflammatory cytokine imbalance in this group compared with normal pregnancies. White blood cells ($p<0.05$; $r=0.345$), and IL-17 ($p<0.05$; $r=0.328$) had weak positive correlation, while CRP ($p<0.01$; $r=0.484$), and IL-6 ($p<0.01$; $r=0.41$), had moderate positive correlation with COVID-19 severity during pregnancy.

Conclusions: COVID-19 infections provide a unique non-dominance of the anti-inflammatory response in pregnant women, as indicated by a lower response of Th2, which may counteract the Th1 response. These cytokine dysregulations may disturb the viral defense mechanism, leading to a proinflammatory condition as shown by higher TNF-α, IL-6, and IFN-γ/IL-4 ratios. Some inflammatory markers have a positive correlation with COVID-19 severity (WBC, CRP, IL-6, and IL-17), suggesting their potential roles as severity markers during pregnancy.

Keywords: COVID-19; cytokines; inflammation; pregnancy.

Resumen

Contexto: Debido a su extensa inflamación, la infección por COVID-19 puede tener una respuesta específica en el medio anti-inflamatorio del embarazo.

Objetivos: Analizar la falta de evidencia de respuestas sistémicas de citocinas inflamatorias y anti-inflamatorias de mujeres embarazadas con infección por COVID-19 en comparación con embarazos normales.

Métodos: Este estudio de casos y controles se realizó en mujeres embarazadas sintomáticas de COVID-19 en el tercer trimestre en comparación con embarazos normales sin otros riesgos significativos de inflamación. Se realizó un análisis entre grupos para evaluar las citocinas y quimiocinas inflamatorias y antiinflamatorias. A través de un análisis de comparación y correlación, detectamos diferencias entre las citocinas en el grupo de COVID-19 en función de la gravedad de la infección.

Resultados: Las gestantes con COVID-19 presentaron niveles más altos de procalcitonina ($p<0,01$), IL-6 ($p=0,05$), TNF-α, relación IFN-γ/IL-4 ($p<0,01$) y menor IL-4 ($p<0,05$), lo que implica un mayor desequilibrio de citocinas proinflamatorias en este grupo en comparación con los embarazos normales. Los glóbulos blancos ($p<0,05$; $r=0,345$) e IL-17 ($p<0,05$; $r=0,328$) tuvieron una correlación positiva débil, mientras que la PCR ($p<0,01$; $r=0,484$) e IL-6 ($p< 0,01$; $r=0,41$), tuvo una correlación positiva moderada con la gravedad de la COVID-19 durante el embarazo.

Conclusiones: Las infecciones por COVID-19 proporcionan una no dominancia única de la respuesta antiinflamatoria en mujeres embarazadas, como lo indica una respuesta más baja de Th2, que puede contrarrestar la respuesta de Th1. Estas desregulaciones de citocinas pueden alterar el mecanismo de defensa viral, lo que lleva a una condición proinflamatoria, como lo demuestran las proporciones más altas de TNF-α, IL-6 e IFN-γ/IL-4. Algunos marcadores inflamatorios tienen una correlación positiva con la gravedad de COVID-19 (WBC, CRP, IL-6 e IL-17), lo que sugiere sus funciones potenciales como marcadores de gravedad durante el embarazo.

Palabras Clave: citocinas; COVID-19; embarazo; inflamación.

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Abbreviations: COVID-19: Coronavirus Disease 2019; CRP: C-reactive protein; ELISA: enzyme-linked immunosorbent assay; IFN: Interferon; IL: Interleukin; IP: Interferon-inducible protein; MCP: Monocyte chemoattractant protein; NLR: Neutrophil to lymphocyte ratio; PCT: procalcitonin; TNF: Tumor necrosis factor; WBC: White Blood Cell Count.

INTRODUCTION

The coronavirus disease (COVID-19) pandemic became the most widespread coronavirus outbreak in history (Feehan and Apostolopoulos, 2021; World Health Organization, 2022). This pandemic affecting the world's population, including the vulnerable pregnant women. Based on prior pandemics, such as the H1N1 and H5N1 outbreaks, which demonstrated substantial morbidity and mortality during pregnancy (Rasmussen et al., 2008; Rasmussen and Jamieson, 2014), it is believed that this demographic necessitates particular care in dealing with COVID-19.

Nevertheless, numerous epidemiological studies have concluded that such complications were discordant with other evidence from the preceding pandemic (Breslin et al., 2020; Wardhana et al., 2021). In contrast, Zambrano's study found a greater need for an intensive care unit (ICU), extracorporeal membrane oxygenation, and invasive breathing in pregnant women despite the low absolute risk of severe output (Zambrano et al., 2020). In order to comprehend how SARS-CoV-2 infection develops in pregnant women, the immunopathology study will be beneficial.

Pregnancy is a unique condition with various adaptations, such as increased immune system tolerance (Ander et al., 2019). The dominance of T helper (Th)-2 over Th1 cytokines as a primary viral immune defense contributes to the vulnerability of pregnant women to complications and mortality when exposed to viral infection (Shah et al., 2019; Silasi et al., 2015). In contrast, COVID-19 has been linked to the emergence of cytokine storm due to Th1 dominance (Huang et al., 2020). There is currently a paucity of data supporting a difference in COVID-19 cytokine production during pregnancy with conflicting findings mostly because of the varied methodologies used, such as the inclusion of non-comparable controls, a wide gestational age, and a small number of samples (Chen G et al., 2021; Garcia-Flores et al., 2022; Tanacan et al., 2021).

To prove the hypothesis of particular alterations in immune dysregulation for COVID-19 in pregnant women, this study restricts samples to only healthy third trimester pregnant women without additional obstetrical or non-obstetrical complications. Consequently, analyzing the precise immune responses and cytokine profiles of pregnant women with COVID-19 will be very important and could help assess their vulnerability (or, conversely, identify protective factors against) COVID-19 resulting from maternal immunological adaptation.

MATERIAL AND METHODS

Subjects and ethics

This case-control study was performed at Dr. Soetomo General Academic Hospital (Surabaya, Indonesia) from December 2020 to May 2021. This study enrolled all pregnant women with symptomatic primary COVID-19 infections who were in the third trimester (gestational age ≥28 weeks). The patients with medical conditions that might cause a cytokine response bias, such as vaccination history, obesity, hypertension in pregnancy, diabetes mellitus, autoimmune disease, renal disease, and other infectious diseases were excluded. COVID-19 was diagnosed by RT-PCR samples collected through oronasopharyngeal swabs. The patients were subsequently followed up until delivery, and the pregnancy outcome data were then collected. As a control group, healthy, COVID-19 negative, third-trimester expectant women who met the same exclusion criteria will be used. The ethical committee of the Dr. Soetomo General Academic Hospital approved this study (No.0099/KEPK/XI/2020). Informed consent was obtained from all the patients before the study began.

Clinical information

All data of maternal age, gestational age, parity, length of stay, and body mass index were taken as basic maternal characteristics. More data for maternal symptoms, symptom onset, ICU admission, ventilator use, and pneumonia appearance in the chest X-Ray examination were added. All maternal blood examinations corresponding to inflammatory parameters were also documented (white blood cell count, platelet count, neutrophils count, lymphocyte count and percentage, neutrophils-to-lymphocyte ratio, procalcitonin, and D-dimer). C-reactive protein (CRP) was also collected in the COVID-19 group. Delivery outcomes (birth weight, and 1- minute Apgar scores) were also taken in the COVID-19 group.

Cytokine analysis

The main outcomes of the study were related to the blood level-based comparisons of inflammatory cytokines, anti-inflammatory, and chemokines between COVID-19 and non-COVID-19 pregnant women. Specifically, the findings regarding TNF-α, IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17, IP-10, MCP-1, and in-

flammatory/anti-inflammatory ratio (IFN-γ/IL-4) were of interest. Twenty millilitres of each patient's blood sample were taken on the first day of admission and then examined in the clinical pathology laboratory of Dr. Soetomo General Academic Hospital. All cytokine levels were measured using flow cytometry methods with a BD™ Cytometric Bead Array / CBA Human Th1/Th2/Th17 cytokine kit (BD Life Sciences – Biosciences) and FCAP Array software, while the levels of IP-10 and MCP-1 were assessed using enzyme-linked immunosorbent assay (ELISA) methods (human IP-10 and human MCP-1 ELISA kit, Bioassay technology laboratory).

This study also performed a subgroup COVID-19 analysis to correlate the inflammatory markers and cytokine-chemokine profiles in primary outcomes with COVID-19 severity during pregnancy. COVID-19 disease severity was categorized as mild, moderate, or severe according to World Health Organization criteria (World Health Organization, 2021). Cases with critical conditions were included in the severe group due to the similar management of severe and critical cases.

The variables used in this study were chemotactic cytokines: IP-10 and MCP-1; inflammatory cytokines: TNF-α, IFN-γ, IL-2, IL-6, and IL-17; anti-inflammatory cytokines: IL-4 and IL-10; the inflammatory/anti-inflammatory ratio: IFN-γ/IL-4 (Fig. 1).

Statistical analysis

The data were analyzed using SPSS ver. 25 (IBM; Armonk, NY, USA). Descriptive statistics were used to assess categorical variables and a chi-square test was used for analysis with Fischer's exact test as an alternative. The numerical variables with normal distributions were analyzed using independent t-test values and are presented as mean ± standard deviation (SD). Meanwhile, the non-normally distributed numerical variables were analyzed using the Mann-Whitney U test and are presented as median values (interquartile range). Multivariable associations between severity levels were analyzed using ANOVA with Kruskal-Wallis test as an alternative. Mann-Whitney U tests were subsequently performed if a significant association was found. The correlation between COVID-19 severity and numerical variables was analyzed using Spearman's rho correlation coefficient. Statistical significance was set to $p<0.05$.

RESULTS

During the study period, 74 pregnant women were enrolled, including 40 women with COVID-19 (54.1%) and 34 pregnant women without COVID-19 (45.9%). No differences were identified between the two groups' basic maternal characteristics and laboratory result, except for longer of hospital stay (p=0.028) and higher procalcitonin (p=0.007) in COVID-19 groups. A summary of the basic characteristics of the participants is presented in Table 1.

According to Fig. 2, the inflammatory and anti-inflammatory cytokines varied between groups, although only the significant differences of higher TNF-α (p=0.006), IL-6 (p=0.05) and lower IL-4 (p=0.012) in COVID-19 pregnant group. There were no significant differences in other inflammatory cytokines, such as IFN-Y, IL-2, IL-17, anti-inflammatory (IL-10) and also chemokines (MCP-1 and IP-10). However, the ratio of IFN-Y to IL-4 is increased overall (p=0.006).

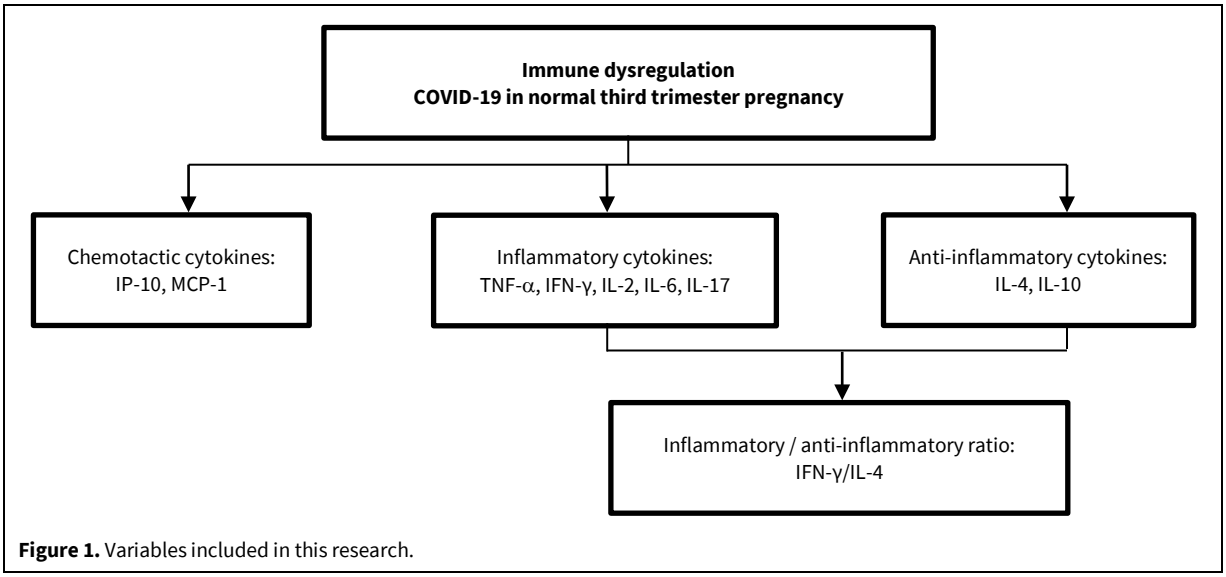

Figure 1. Variables included in this research.

Table 1. Basic characteristics of pregnant women in the study.

Characteristics	Normal pregnancy (n = 34)	Pregnancy with COVID-19 (n = 40)	p-value
Basic maternal characteristics			
Maternal age (y.o)	32 (7)	29.5 (7)	0.463
Gestational age (weeks)	36 (4.25)	36 (5)	0.460
Parity			
Primigravida	5 (14.7%)	14 (35%)	0.063
Multigravida	29 (85.3%)	26 (65%)	
Length of stay (days)	4.5 (2.25)	6 (8.75)	**0.028**
Body mass index (kg/m^2)	25.8 ± 4.09	25.04 ± 3.65	0.399
Laboratory results			
Hemoglobin (g/dL)	10.56 ± 1.57	10.80 ± 1.29	0.465
White blood cell count (× 10^3/µL)	10.24 (3.21)	9.3 (3.78)	0.310
Platelet (× 10^3/µL)	293.00 ± 62.83	274.42 ± 89.5	0.272
Neutrophils count (× 10^3/µL)	8.65 (7.03)	7.54 (4.48)	0.062
Lymphocyte count (× 10^3/µL)	1.65 ± 0.61	1.42 ± 0.56	0.094
Neutrophils-to-lymphocyte ratio	4.92 (5.25)	5.82 (4.80)	0.996
Procalcitonin (ng/mL)	0.08 (0.17)	0.2 (0.24)	**0.007**
D-Dimer (ng/mL)	1,757 (1,795)	2,150 (3,310)	0.125

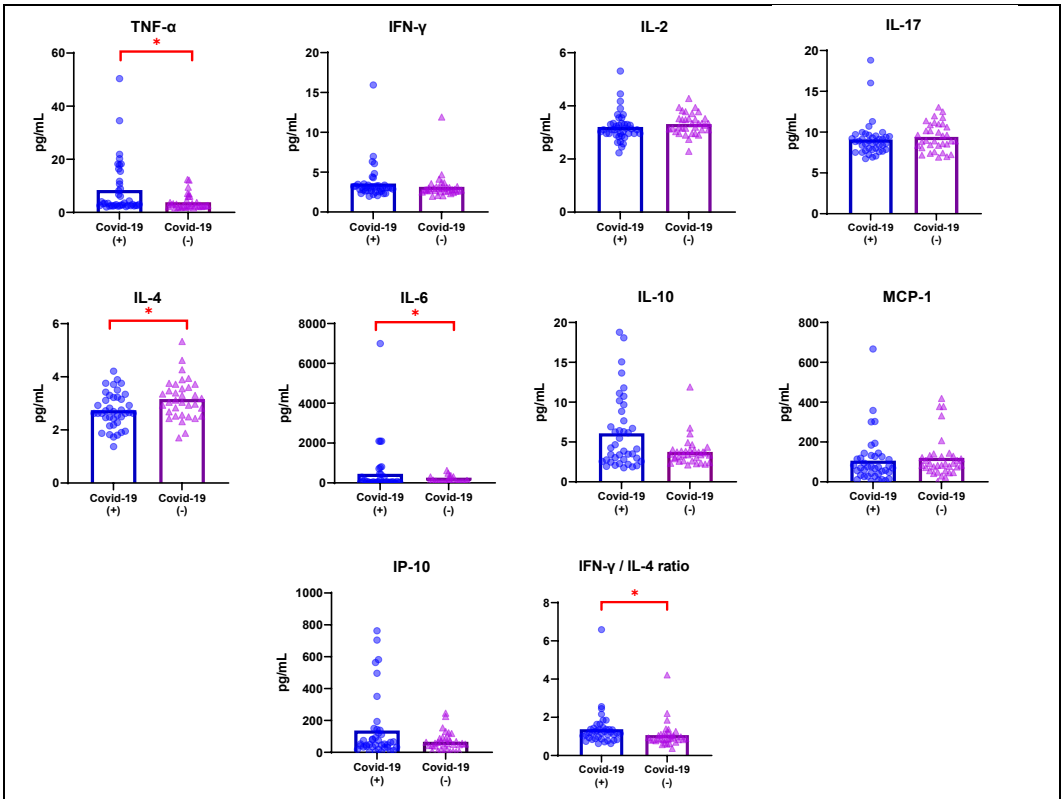

Figure 2. Plasma levels comparison of cytokines and chemokines between pregnant women with COVID-19 (n = 40) and normal pregnant women (n = 34).

Red lines and * above the columns indicated significant differences between the two columns.

Table 2. Characteristics of pregnant women with COVID-19 based on disease severity.

Characteristics	Mild (n = 13)	Moderate (n = 13)	Severe (n = 14)	p-value
Basic maternal characteristics				
Maternal age (y.o)	27.30 ± 5.19	31.70 ± 3.77	32.00 ± 4.31	0.086
Gestational age (weeks)[a]	35.23 ± 2.86*	37.08 ± 1.50^	33.07 ± 3.12*^	**0.004**
Parity				
primigravida	4 (30.8%)	7 (53.8%)	3 (21.4%)	0.195
multigravida	9 (69.2%)	6 (46.2%)	11 (78.6%)	
Symptoms				
Common cold	8 (61.5%)	1 (7.7%)	1 (7.7%)	**0.001**
Anosmia	2 (15.4%)	3 (23.1%)	2 (14.3%)	0.810
Cough	6 (46.2%)	12 (92.3%)	14 (100%)	**0.001**
Febrile	1 (7.7%)	2 (15.4%)	5 (35.7%)	0.168
Shortness of breath	0 (0%)	1 (7.7%)	13 (92.9%)	**<0.001**
Symptom onset	4 (2)	3 (1)	4 (2)	0.099
Length of stay (days)	7 (8.5)	7 (9.5)	3.5 (9.25)	0.406
Intensive Care Unit admission	0 (0%)	0 (0%)	9 (64.3%)	**<0.001**
Ventilator use	0 (0%)	0 (0%)	7 (50%)	**<0.001**
Body mass index (kg/m^2)	24.78 ± 4.12	25.68 ± 3.47	24.69 ± 3.54	0.754
Pneumonia (chest X-Ray)	0 (0%)	13 (100%)	14 (100%)	**<0.001**
Laboratory results				
Haemoglobin (g/dL)	10.18 ± 1.66	10.99 ± 0.99	11.19 ± 0.97	0.100
White blood cell count (× 10^3/µL)	8.25 (2.66)	9.64 (3.4)	11.03 (6.48)	0.096
Platelet (× 10^3/µL)	255.31 ± 70.21	261.46 ± 61.31	304.21 ± 95.71	0.097
Neutrophils count (× 10^3/µL)	6.56 (2.53)	7.69 (2.68)	9.09 (7.40)	0.097
Lymphocyte count (× 10^3/µL)	1.25 ± 0.41	1.58 ± 0.73	1.42 ± 0.48	0.324
Neutrophils-to-lymphocyte ratio	4.67 (2.33)	5.43 (3.86)	8.31 (5.65)	0.213
Procalcitonin (ng/mL)	0.16 ± 0.10	0.17 ± 0.13	0.25 ± 0.16	0.151
C-Reactive Protein (mg/L)[b]	0.6 (3.25)*	0.5 (0.85)^	4.5 (8.28)*^	**0.001**
D-Dimer (ng/mL)	2,743.85 (2,860)	3,520 (3,610)	2,290 (3,817.5)	0.591
Delivery outcomes				
Birth weight (g)[c]	2793.85 ± 612.50*	3115.38 ± 430.30^	2061.54 ±728.91*^	**<0.001**
Apgar score 1 min[d]	6 (2)	7 (1.5)*	5 (5)*	**0.042**

^°*: significant pairwise comparisons between two groups- [a]Gestational age: * P=0.017; ^ P=0.001. [b]C-Reactive protein: * P=0.003; ^ P<0.001. [c]Birth weight: * P=0.011; ^ P<0.001. [d]Apgar score 1 minute: * p=0.026. The severity of disease divided into mild: symptomatic patients without evidence of viral pneumonia or hypoxia; moderate: clinical sign of pneumonia but no sign of severe pneumonia including $SpO_2 \geq 90\%$ on room air; and severe: clinical sign of pneumonia plus one of the following (respiratory rate > 30 breaths / min), severe respiratory distress, or $SpO_2 < 90\%$ on room air (World Health Organization, 2021). The disease severity in the present study was classified according to WHO guideline, which mainly assessed patient's condition based on sign of respiratory distress, pneumonia, and oxygen saturation on room air (Living guidance for clinical management of COVID-19, https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2).

In a subgroup analysis of 40 COVID-19 pregnant women (54.1%), mild and moderate severity occurred in 13 cases each, and 14 cases of severe COVID-19 were reported (Table 2). Concerning the effects of COVID-19 on pregnancy, pregnant women who experienced severe COVID-19 symptoms had significantly higher rates of ICU admission, ventilator use, pneumonia (p<0.001), and preterm birth (p=0.004). Additionally, one patient (7.1%) with severe symptoms died due to COVID-19 complications. The laboratory results presented no significant differences between groups, except that CRP levels were higher in patients with severe symptoms (p=0.001). There were significant differences in neonatal outcomes, as severe

symptoms associated with lower birth weight (p<0.001) and Apgar scores (1-minute: p=0.042; 5-minutes: p=0.025). Table 2 summarizes the basic characteristics of pregnant women infected with COVID-19.

In addition, this study performed a correlation analysis of all cytokine markers and COVID-19 severity (Fig. 3). The COVID-19 severity in pregnant woman was positively correlated with WBC, CRP, IL-6, and IL-17 levels. No other significant correlations were detected (Table 3).

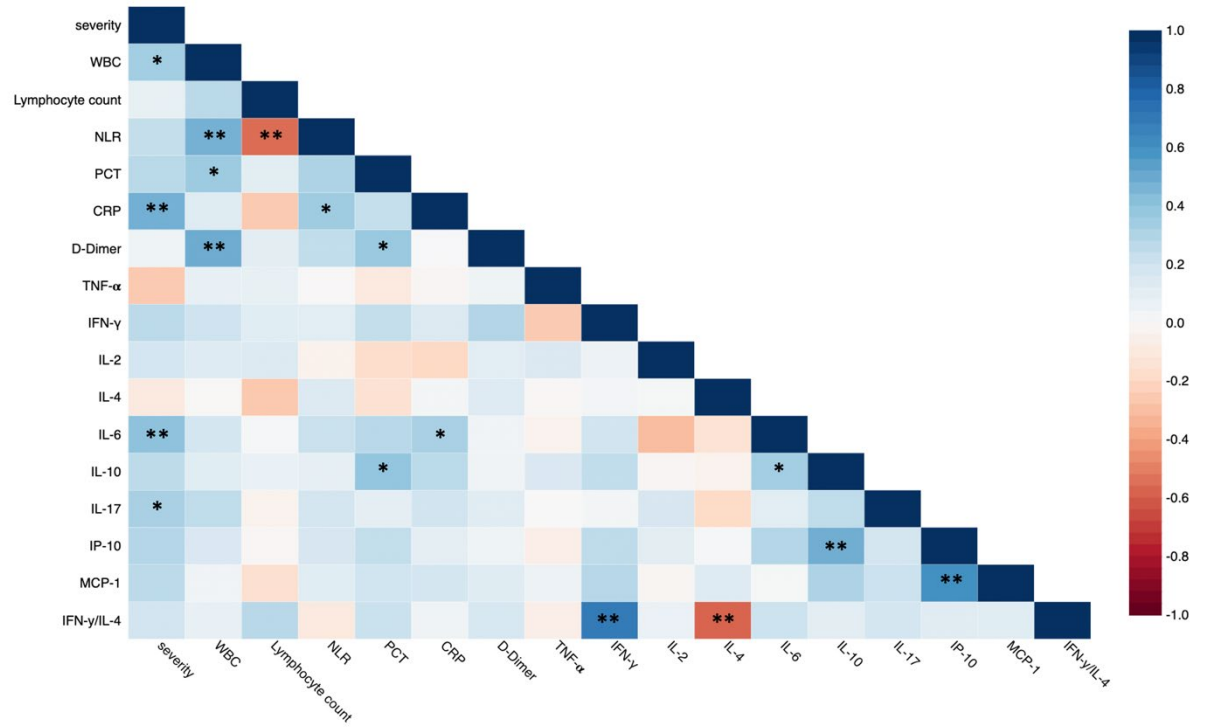

Figure 3. Correlation matrix of severity and cytokine markers in pregnant women with COVID-19.

Significance for the strength and direction of correlation between the two variables indicated in each matrix were determined using the Spearman rho correlation coefficient. *p<0.05 and ** p<0.01.

Table 3. Correlation of inflammation, cytokine, and chemokine parameter with COVID-19 infection severity in pregnant women

Serum inflammation parameter	Correlation coefficient	p value	Cytokine and chemokine parameter	Correlation coefficient	p value
WBC	0.345	**0.029**	IFN-γ	0.269	0.093
Lymphocyte count	0.083	0.61	TNF-α	-0.262	0.103
NLR	0.243	0.131	IL-2	0.185	0.252
PCT	0.276	0.085	IL-4	-0.096	0.554
CRP	0.484	**0.002**	IL-6	0.41	**0.009**
D-Dimer	0.05	0.76	IL-10	0.265	0.098
			IL-17	0.328	**0.039**
			IP-10	0.294	0.066
			MCP-1	0.27	0.092
			IFN-γ/IL-4	0.198	0.22

WBC: White Blood Cell Count; NLR: Neutrophil to lymphocyte ratio, PCT: procalcitonin, CRP: C-reactive protein, TNF: Tumor necrosis factor, IFN: Interferon, IL: Interleukin, IP: Interferon-inducible protein, MCP: Monocyte chemoattractant protein.

DISCUSSION

To investigate the specific immunological responses of pregnancies affected by COVID-19, the cytokine profiles of pregnant women with and without COVID-19 were evaluated in this study. While the immunopathogenesis of COVID-19 is well-developed in the general population, it is unknown whether this is the case among pregnant women due to contradictory evidence (Chen G et al., 2021; Tanacan et al., 2021). The current study included only pregnant women in their third trimester because variations in immunological adaptability and cytokine production across the trimesters (Lim et al., 2020). Moreover, this research omitted multiple medical conditions, including preeclampsia, which enhances the systemic Th1/Th2 ratio (Raghupathy and Szekeres-Bartho, 2022; Szarka et al., 2010), so that, the COVID-19 specific immunological changes can be more properly identified.

There is no difference in the characteristic, particularly in gestational age and body mass index, could have affected the outcomes. The increased hospitalization was likely due to the official isolation period protocol (Burhan et al., 2020). Procalcitonin is an excellent inflammatory diagnostic marker and is produced by nearly all cell types (Mangogna et al., 2019). Procalcitonin can be indirectly stimulated by proinflammatory cytokines, some of which were observed at increased levels in our study. Nonetheless, these increases can be mitigated by the release of IFN-γ in response to viral infection (Hu et al., 2020; Meili et al., 2015). Thus, the value would rarely exceed 0.5 ng/mL, which is consistent with the findings of our study.

The current study demonstrated that COVID-19 infection is primarily associated with inflammatory and anti-inflammatory cytokine responses (Tay et al., 2020). Local inflammation enhances the secretion of proinflammatory cytokines and chemokines, such as TNF-α, IL-1β, IL-6, IFN-γ, MCP-1, and IP-10, into the systemic circulation, which characterizes the Th1 cell response polarization (Huang et al., 2020). In SARS-CoV-2 infection, however, Th2 cytokine release (such as IL-4 and IL-10) typically increases, which makes it distinct from prior SARS-CoV infections (Han et al., 2020; Huang et al., 2020; Tay et al., 2020). Similarly, to the general population, this study revealed higher levels of TNF-α and IL-6 in infected COVID-19 pregnant women. However, these results contradict other previous study. Tanacan et al. (2021) discovered greater IL-6 levels in a maternal COVID-19 group overall but not in the third trimester. Other studies also found no differences between these proinflammatory cytokines (Chen G et al., 2021; Garcia-Flores et al., 2022), which may because of different methodological study; for instance, the fewer, milder/asymptomatic cases and some other inflammatory pathology condition in pregnancy that can interfere immunopathological reactions (Raghupathy and Szekeres-Bartho, 2022; Szarka et al., 2010).

Intriguingly, authors observed decreased of anti-inflammatory IL-4 and a non-significant rise in IL-10. However, during pregnancy, both are frequently expressed at substantial concentrations and as previously reported, COVID-19 infections boost the Th2 cytokines expression in the general population (Huang et al., 2020). Previous pregnancy-specific research has yielded inconclusive results. Chen G et al. (2021) did not find a significant change in IL-4, whereas Tanacan et al. (2021) observed a decrease in IL-10 secretion and Garcia-Flores et al. (2022) studied the opposite. The results of this investigation demonstrate that the Th2 predominance during normal pregnancy shifts to Th1 during COVID-19 infections. This shift also occurs in certain pregnancy-related pathologies, such as preeclampsia (Jonsson et al., 2006). The decreased production of IL-4 and increased ratio of Th1/Th2 (IFN-γ/IL-4) in COVID-19-infected pregnant women corroborated these postulates.

Pregnancy is characterized by progesterone dominance and its immunomodulatory molecules (Progesterone induced Blocking Factor), which induces numerous alterations in immune responses, specifically preventing the production of Th1 cytokines (Shah et al., 2019). This study revealed an absence of enhanced IFN-γ expression, which plays a vital role in defense against viruses, which may increase SARS-CoV-2 infection susceptibility, severity and impair their ability for virus-infected cells clearance (Chen R et al., 2021; Dashraath et al., 2020). Hu's study revealed decreased circulating IFN-γ is a risk factor for pulmonary fibrosis among COVID-19 patients (Hu et al., 2020). Therefore, the low IFN-γ response shown in this study, which is likely attributable to Th2 suppression during pregnancy (Raghupathy and Szekeres-Bartho, 2022), can render COVID-19-infected pregnancy hazardous.

Maternal adaptation can affect numerous other inflammatory parameters, no significant differences in the levels of other inflammatory markers were observed in this study. Although there is insufficient information on the effects of SARS-CoV-2 infection on pregnant women's cytokine profiles, the aforementioned data could indicate an altered immune response in maternal circulation (Miller et al., 2020; Lee et al., 2014).

Subgroup analysis was also performed to better understand the severity differences in COVID-19 immunopathological changes in pregnancy. This

study discovered no significant differences in the onset of symptoms, which could influence different cytokine release (He et al., 2020). More severe prematurity-related conditions may be associated with low birth weight and poor neonatal outcomes. This condition has also been identified in prior studies (Metz et al., 2021; Vouga et al., 2021). More severe conditions prompt individuals to visit health facilities, where iatrogenic labor may be required due to serious respiratory disorders (Debrabandere et al., 2021). This study also found that maternal pneumonia occurrences, critical care, and ventilator requirements are associated with more severe conditions.

Previous research demonstrated that IL-6 increases the severity of COVID-19, particularly among non-survivors (Zhou et al., 2020). Other results revealed a similar moderate positive correlation of severity for both IL-6 and CRP with this study. IL-6 is linked to the acute phase response, and its plasma concentrations are prognostic of prolonged mechanical ventilation, organ dysfunction, and mortality in pulmonary disease. As such, the excessive secretion of IL-6 in response to COVID-19 results in a cytokine storm (Chen LD et al., 2020; Han et al., 2020). A weak correlation between IL-17 and COVID-19 severity in pregnancy was also found. IL-6 is also highly engaged in the induction of Th17, and the cytokine produced (IL-17) works synergistically with IL-6 to induce an inflammatory response that can cause respiratory system injuries (Megna et al., 2020; Sadeghi et al., 2020). Notably, IL-17 can cause severe lung injuries by altering epithelial cell apoptosis, which encourages fibroblast conversion and results in pulmonary fibrosis (Gouda and Bhandary, 2018).

Immune responses through the recruitment and clearance of immune cells, the interplay between IFN-γ and IL-6 is crucial for regulating infections and acute inflammation. Higher levels of IL-6 are associated with an increased mortality risk, whereas IFN-γ expression tends to be lower in severe cases than in mild cases (Chen G et al., 2020; Ruan et al., 2020). This condition is supported by the present study's findings that IL-6 increased while IFN-γ did not. Another study compared the ratio of IL-6 to IFN-γ and revealed that this ratio was higher in COVID-19 patients with severe diseases than those with milder diseases (Lagunas-Rangel and Chávez-Valencia, 2020). These conditions show the importance of an effective IFN response for COVID-19 prognosis, which also indicates its possible role in therapy for viral suppression (Lopez et al., 2020).

The current study cannot represent the entire gestation period because only pregnant women in the third trimester were considered, as most cases that came to the hospital were in the late pregnancy period. Therefore, knowledge of immunopathological changes in this period is required. Furthermore, this study was limited to a single institution. Nevertheless, it has the advantage of using stringent inclusion and exclusion criteria to eliminate immunological bias for various reasons. In addition, in this study, sample tests were performed upon the patient's admission, which could have impacted the condition of the cytokines produced as the infection progressed. Apart from these limitations, this study adds to the growing evidence showing that because pregnancy involves anti-inflammatory dominance, the proinflammatory conditions required for viral eradication, including in COVID-19, can be suppressed. Thus, pregnant women may appear to be vulnerable to COVID-19.

CONCLUSION

COVID-19 infections during pregnancy provide specific and unique changes in immunopathology. Anti-inflammatory dominance during pregnancy does not occur during COVID-19 infections, as demonstrated by the weakened Th2 (IL-4) response that could successfully suppress the Th1 response (a non-significant increase in IFN-γ). These cytokine dysregulations may disrupt the viral defense mechanism, resulting in a proinflammatory state characterized by higher levels of TNF-α, IL-6, and a higher IFN-γ/IL-4 ratio. However, only WBC, CRP, IL-6, and IL-17 were positively correlated with COVID-19 severity during pregnancy, indicating their potential as severity biomarkers. Nevertheless, further studies are needed to corroborate these findings.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Ander SE, Diamond MS, Coyne CB (2019) Immune responses at the maternal-fetal interface. Sci Immunol 4(31): eaat6114. https://doi.org/10.1126/sciimmunol.aat6114

Breslin N, Baptiste C, Gyamfi-Bannerman C, Miller R, Martinez R, Bernstein K, Ring L, Landau R, Purisch S, Friedman AM, Fuchs K, Sutton D, Andrikopoulou M, Rupley D, Sheen JJ, Aubey J, Zork N, Moroz L, Mourad M, Wapner R, Simpson LL, D'Alton ME, Goffman D (2020) Coronavirus disease 2019 infection among asymptomatic and symptomatic pregnant women: two weeks of confirmed presentations to an affiliated pair of New York City hospitals. Am J Obstet Gynecol MFM 2(2): 100118. https://doi.org/10.1016/J.AJOGMF.2020.100118

Burhan E, Susanto AD, Isbaniah F, Nasution SA, Ginanjar E, Pitoyo CW, Susilo A, Firdaus I, Santoso A, Juzar DA, Arif SK,

Wulung NGL, Muchtar F, Pulungan AB, Sjakti HA, Prawira Y, Putri ND (2020) Pedoman Tatalaksana COVID-19 3rd ed. Jakarta: PDPI, PERKI, PAPDI, PERDATIN, IDAI.

Chen G, Liao Q, Ai J, Yang B, Bai H, Chen J, Liu F, Cao Y, Liu H, Li K (2021) Immune response to COVID-19 during pregnancy. Front Immunol 12: 675476. https://doi.org/10.3389/fimmu.2021.675476

Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, Wang T, Zhang X, Chen H, Yu H, Zhang X, Zhang M, Wu S, Song J, Chen T, Han M, Li S, Luo X, Zhao J, Ning Q (2020) Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest 130(5): 2620–2629. https://doi.org/10.1172/JCI137244

Chen LD, Zhang ZY, Wei XJ, Cai YQ, Yao WZ, Wang MH, Huang QF, Zhang XB (2020) Association between cytokine profiles and lung injury in COVID-19 pneumonia. Respir Res 21(1): 201. https://doi.org/10.1186/s12931-020-01465-2

Chen R, Zhang S, Su S, Ye H, Shu H (2021) Interactions between specific immune status of pregnant women and SARS-CoV-2 infection. Front Cell Infect Microbiol 11: 721309. https://doi.org/10.3389/fcimb.2021.721309

Dashraath P, Wong JLJ, Lim MXK, Lim LM, Li S, Biswas A, Choolani M, Mattar C, Su LL (2020) Coronavirus disease 2019 (COVID-19) pandemic and pregnancy. Am J Obstet Gynecol 222(6): 521–531. https://doi.org/10.1016/j.ajog.2020.03.021

Debrabandere ML, Farabaugh DC, Giordano C (2021) A review on mode of delivery during COVID-19 between December 2019 and April 2020. Am J Perinatol 38(4): 332–341. https://doi.org/10.1055/s-0040-1721658

Feehan J, Apostolopoulos V (2021) Is COVID-19 the worst pandemic? Maturitas 149: 56–58. https://doi.org/10.1016/j.maturitas.2021.02.001

Garcia-Flores V, Romero R, Xu Y, Theis KR, Arenas-Hernandez M, Miller D, Peyvandipour A, Bhatti G, Galaz J, Gershater M, Levenson D, Pusod E, Tao L, Kracht D, Florova V, Leng Y, Motomura K, Para R, Faucett M, Hsu CD, Zhang G, Tarca AL, Pique-Regi R, Gomez-Lopez N (2022) Maternal-fetal immune responses in pregnant women infected with SARS-CoV-2. Nat Commun 13: 320. https://doi.org/10.1038/s41467-021-27745-z

Gouda MM, Bhandary YP (2018) IL-17A mediated downstream changes during acute lung injury keywords. iMedPub Journals 4(2): 1–3. https://doi.org/10.21767/2471-8084.100062

Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, Liu X, Gao G, Liu F, Jiang Y, Zhu C, Xia Y (2020) Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg Microbes Infect 9(1): 1123–1130. https://doi.org/10.1080/22221751.2020.1770129

He X, Lau EHY, Wu P, Deng X, Wang J, Hao X, Lau YC, Wong JY, Guan Y, Tan X, Mo X, Chen Y, Liao B, Chen W, Hu F, Zhang Q, Zhong M, Wu Y, Zhao L, Zhang F, Cowling BJ, Li F, Leung GM (2020) Temporal dynamics in viral shedding and transmissibility of COVID-19. Nat Med 26(5): 672–675. https://doi.org/10.1038/S41591-020-0869-5

Hu R, Han C, Pei S, Yin M, Chen X (2020) Procalcitonin levels in COVID-19 patients. Int J Antimicrob Agents 56(2): 106051. https://doi.org/10.1016/j.ijantimicag.2020.106051

Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395(10223): 497–506. https://doi.org/10.1016/S0140-6736(20)30183-5

Jonsson Y, Rub M, Nieminen K, Sharma S, Ernerudh J, Ekerfelt C (2006) Cytokine mapping of sera from women with preeclampsia and normal pregnancies. J Reprod Immunol 70(1–2): 83–91. https://doi.org/10.1016/j.jri.2005.10.007

Lagunas-Rangel FA, Chávez-Valencia V (2020) High IL-6/IFN-γ ratio could be associated with severe disease in COVID-19 patients. J Med Virol 92(10): 1789–1790. https://doi.org/10.1002/jmv.25900

Lee GH, Chan Y, Kim TJ (2014) Immune mechanisms at the maternal-fetal interface: perspectives and challenges. Ultrasmall Lanthan Oxide Nanoparticles Biomed Imaging Ther 16(4): 328–334. https://doi.org/10.1533/978008100694.103

Lim MK, Ku CW, Tan TC, Hao Y, Lee J, Allen JC, Tan NS (2020) Characterisation of serum progesterone and progesterone-induced blocking factor (PIBF) levels across trimesters in healthy pregnant women. Sci Rep 10: 3840. https://doi.org/10.1038/s41598-020-59452-y

Lopez L, Sang PC, Tian Y, Sang Y (2020) Dysregulated interferon response underlying severe COVID-19. Viruses 12(12): 1433. https://doi.org/10.3390/V12121433

Mangogna A, Agostinis C, Ricci G, Romano F, Bulla R (2019) Overview of procalcitonin in pregnancy and in pre-eclampsia. Clin Exp Immunol 198(1): 37–46. https://doi.org/10.1111/CEI.13311

Megna M, Napolitano M, Fabbrocini G (2020) May IL-17 have a role in COVID-19 infection? Med Hypotheses 140: 109749. https://doi.org/10.1016/j.mehy.2020.109749

Meili M, Müller B, Kulkarni P, Schütz P (2015) Management of patients with respiratory infections in primary care: procalcitonin, C- reactive protein or both? Expert Rev Respir Med 9(5): 587–601. https://doi.org/10.1586/17476348.2015.1081063

Metz TD, Clifton RG, Hughes BL, Sandoval G, Saade GR, Grobman WA, Manuck TA, Miodovnik M, Sowles A, Clark K, Gyamfi-Bannerman C, Mendez-Figueroa H, Sehdev HM, Rouse DJ, Tita ATN, Bailit J, Costantine MM, Simhan HN, Macones GA (2021) Disease severity and perinatal outcomes of pregnant patients with coronavirus disease 2019 (COVID-19). Obstet Gynecol 137(4): 571–580. https://doi.org/10.1097/AOG.0000000000004339

Miller D, Gershater M, Slutsky R, Romero R, Gomez-Lopez N (2020) Maternal and fetal T cells in term pregnancy and preterm labor. Cell Mol Immunol 17(7): 693–704. https://doi.org/10.1038/s41423-020-0471-2

Raghupathy R, Szekeres-Bartho J (2022) Progesterone: A unique hormone with immunomodulatory roles in pregnancy. Int J Mol Sci 23(3): 1333. https://doi.org/10.3390/ijms23031333

Rasmussen SA, Jamieson DJ (2014) 2009 H1N1 Influenza and pregnancy — 5 years later. N Engl J Med 371(15): 1373–1375. https://doi.org/10.1056/NEJMp1403496

Rasmussen SA, Jamieson DJ, Bresee JS (2008) Pandemic influenza and pregnant women. Emerg Infect Dis 14(1): 95–100. https://doi.org/10.3201/eid1401.070667

Ruan Q, Yang K, Wang W, Jiang L, Song J (2020) Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med 46(5): 846–848. https://doi.org/10.1007/s00134-020-05991-x

Sadeghi A, Tahmasebi S, Mahmood A, Kuznetsova M, Valizadeh H, Taghizadieh A, Nazemiyeh M, Aghebati L, Farhad M, Niaragh J, Abbaspour S, Mikaeili H, Ahmadi M, Roshangar L (2020) Th17 and Treg cells function in SARS-CoV2 patients compared with healthy controls. J Cell Physiol 236(4): 2829–2839. https://doi.org/10.1002/jcp.30047

Shah NM, Lai PF, Imami N, Johnson MR (2019) Progesterone-related immune modulation of pregnancy and labor. Front Endocrinol 10(3): 198. https://doi.org/10.3389/fendo.2019.00198

Silasi M, Cardenas I, Kwon J young, Racicot K, Aldo P, Mor G (2015) Viral infections during pregnancy. Am J Reprod Immunol 73(3): 199–213. https://doi.org/10.1111/aji.12355

Szarka A, Jr JR, Lázár L, Bek G, Molvarec A (2010) Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. BMC Immunol 11: 59. https://doi.org/10.1186/1471-2172-11-59

Tanacan A, Yazihan N, Ahmet S, Taner A (2021) The impact of COVID-19 infection on the cytokine profile of pregnant women: A prospective case-control study. Cytokine 140(1): 155431. https://doi.org/10.1016/j.cyto.2021.155431

Tay MZ, Poh CM, Rénia L, Macary PA, Ng LFP (2020) The trinity of COVID-19: Immunity, inflammation and intervention. Nat Rev Immunol 20(6): 363–374. https://doi.org/10.1038/s41577-020-0311-8

Vouga M, Favre G, Perez OM, Pomar L, Acebal LF, Saiz AA, Rosa M, Hernandez V, Hcini N, Lambert V, Carles G, Sichitiu J, Salomon L, Stirnemann J, Ville Y, Tejada BM De, Goncé A, Villarreal AH, Castillo K, Solsona EG, Trigo L (2021) Maternal outcomes and risk factors for COVID-19 severity among pregnant women. Sci Rep 11(1): 13898. https://doi.org/10.1038/s41598-021-92357-y

Wardhana MP, Maniora NC, Aditya R, Gumilar, K.E. Wicaksono B, Akbar MI, Sulistyono A, Juwono HT, Dachlan EG (2021) SARS-CoV-2 antibody test for the hospitalised emergency obstetric cases: Useful or wasteful. New Armen Med J 15(2): 56–63.

World Health Organization (2021) COVID-19 clinical management: living guidance.

World Health Organization (2022) WHO Corona Virus (COVID-19) Dashboard: Situation by Region, Country, Territory & Area.

Zambrano LD, Ellington S, Strid P, Galang RR, Oduyebo T, Tong VT, Woodworth KR, Nahabedian JF 3rd, Azziz-Baumgartner E, Gilboa SM, Meaney-Delman D; CDC COVID-19 Response Pregnancy and Infant Linked Outcomes Team (2020) Update: Characteristics of symptomatic women of reproductive age with laboratory-Confirmed SARS-CoV-2 infection by pregnancy status - United States, January 22-October 3, 2020. MMWR Morb Mortal Wkly Rep 69(44): 1641–1647. https://doi.org/10.15585/mmwr.mm6944e3

Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B (2020) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 395(10229): P1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3

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Contribution	Wardhana MP	Dachlan EG	Kuntaman K	Utomo B	Wafa IA	Rifdah SN	Banjarnahor DPP	Danianto A
Concepts or ideas	x							
Design	x	x	x	x				
Definition of intellectual content	x	x	x	x				
Literature search	x				x	x	x	x
Data acquisition	x						x	x
Data analysis	x							
Statistical analysis	x							
Manuscript preparation	x	x	x	x	x	x		
Manuscript editing	x				x	x		
Manuscript review	x	x	x	x	x	x	x	x

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