

# Description of Neutrophil-to-lymphocyte Ratio, C-reactive Protein, and Procalcitonin Levels in Pregnancy with COVID-19 at Sanglah General Hospital Period of April 2020–April 2021

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## ABSTRACT

**Background:** Theoretically, pregnant women are more susceptible to infection of coronavirus disease 2019 (COVID-19) and severe pneumonia due to presentation of physiological changes adaptation and immunosuppression during pregnancy. Based on the immune clock theory, if pregnant women had COVID-19 in the first and third it can appearance of cytokine storm due to hyperinflammation state, and lead to poor maternal and neonatal outcomes. Hyperinflammation state is characterized by an increase in inflammatory biomarkers in the serum, including the neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), and procalcitonin levels. This study aims to determine the characteristic of NLR, CRP, and procalcitonin in pregnancy with COVID-19 at Sanglah General Hospital, Denpasar, Bali, Indonesia.

**Methods:** This study is a cross-sectional descriptive study using secondary data from patient's medical records and is conducted in the delivery room and medical record department at Sanglah General Hospital, Denpasar from April 2020 to April 2021.

**Result:** Pregnancy cases with COVID-19 in this study were in the 26–30-year age group, as much as 26 cases (37.14%) with most common comorbidities found were preeclampsia and electrolyte imbalance, as much as nine cases each (12.86%). Most of the neonates born at term, with birth weight 2,500 gm, and vigorous baby. The highest median NLR value was found in pregnant women with COVID-19 in comorbid with obesity, 6.79 (2.88–9.14). In this study, the cases with a length of stay more than or 10 days had a median NLR value of 6.93 (2.57–20.69), CRP 59.83 (1.60–151.56), and procalcitonin 0.145 (0.03–2.56), which are all higher than those whose length of stay was less than 10 days.

**Conclusion:** NLR, CRP, and procalcitonin values are affected by the trimester of pregnancy, the maternal comorbidities, the commonly found chest X-ray features, the length of hospital stay, the prognostic value to be determined to find the disease severity, the needs of oxygen supplementation, and the intensive care treatment, and also they could be the predictors for neonatal outcome in pregnancy with COVID-19.

**Keywords:** COVID-19 in pregnancy, C-reactive protein, Neutrophil-to-lymphocyte ratio, Pregnancy.

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## INTRODUCTION

Pregnant women are more susceptible to COVID-19 and severe pneumonia, due to changes in physiological adaptation and immunosuppression during pregnancy.<sup>1</sup> The immunological peculiarities where there is a change from a proinflammatory state in the first trimester to an anti-inflammatory state in the second trimester and a return to a proinflammatory state in the third trimester needs to be watched out for because the proinflammatory state in the first and third trimesters, if exacerbated by cytokine storm due to COVID-19, can cause poor maternal and neonatal outcomes, including premature rupture of membranes, premature birth, fetal distress, and the risk of vertical transmission from mother to fetus.<sup>2</sup> Cytokine storm induced by COVID-19 is characterized by elevated serum cytokines, hyperinflammation, and cytopenia. Hyperinflammation is characterized by an increase in inflammatory biomarkers in the serum, including the NLR, CRP, and procalcitonin levels. This study aims to determine the characteristic of NLR, CRP, and procalcitonin in pregnancy with COVID-19 at Sanglah General Hospital, Denpasar, Bali, Indonesia.

## METHODS

This study is a cross-sectional descriptive study using secondary data from patient's medical records and is conducted in the delivery room and medical record department at Sanglah General Hospital, Denpasar from April 2020 to April 2021. The sample of this study was all pregnant women with COVID-19 at Sanglah General

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Hospital, Denpasar from April 2020 to April 2021, and has complete data of NLR, CRP, and Procalcitonin. A total of 70 pregnant women with COVID-19 met the criteria of this study. All data in this study is processed and tabulated using Microsoft Excel and SPSS, version 25, software. The data are presented as frequency distributions and percentages, and also as medians with interquartile ranges.

## RESULTS

In the period April 2020–April 2021, there were 154 pregnant women with COVID-19 who were treated at Sanglah General Hospital, but only 70 patients (45.45%) had complete data of NLR, CRP, and procalcitonin levels and were included in this study.

**Table 1:** Characteristic distribution of pregnancy with COVID-19

		N	%
Maternal age	15–20-year old	3	4.29
	21–25-year old	11	15.71
	26–30-year old	26	37.14
	31–35-year old	17	24.29
	36–40-year old	12	17.14
	>40 years	1	1.43
	<b>Total</b>	<b>70</b>	<b>100</b>
Referral origin	Non-referral case	26	37.14
	Public hospital	17	24.29
	Private hospital	20	28.56
	Midwife	3	4.29
	Public health office	1	1.43
	Udayana University Hospital	2	2.86
	O&G	1	1.43
	<b>Total</b>	<b>70</b>	<b>100</b>
Parity	0	17	24.29
	1	27	38.57
	2	18	25.71
	3	8	11.43
	<b>Total</b>	<b>70</b>	<b>100</b>
Gestational age	First trimester	3	4.29
	Second trimester	7	10.00
	Third trimester	60	85.71
	<b>Total</b>	<b>70</b>	<b>100</b>
Maternal comorbidity	No comorbidity	42	60.00
	Preeclampsia	9	12.86
	Heart failure	1	1.43
	Electrolyte imbalance	9	12.86
	Thrombocytopenia	4	5.71
	Hepatitis B	1	1.43
	Obesity	4	5.71
	<b>Total</b>	<b>70</b>	<b>100</b>
	Length of stay	<10 days	38
≥10 days		32	45.71
<b>Total</b>		<b>70</b>	<b>100</b>
Chest X-ray feature	Pneumonia	50	81.97
	Without pneumonia	11	18.03
	<b>Total</b>	<b>61*</b>	<b>100</b>
Symptoms	Asymptomatic	21	30.00
	Mild symptoms	13	18.57
	Moderate symptoms	16	22.86
	Severe symptoms	8	11.43
	Critically ill	12	17.14
	<b>Total</b>	<b>70</b>	<b>100</b>

(Contd...)

**Table 1: (Contd...)**

		N	%
Oxygen supplementation	Without oxygen supplementation	42	60.00
	Nasal canule	10	14.29
	Face mask	2	2.86
	Non-rebreathing mask	5	7.14
	High flow nasal canule	1	1.42
	Ventilator	10	14.29
	<b>Total</b>	<b>70</b>	<b>100</b>
Needs of intensive care	Intensive care	14	20.00
	Without intensive care	56	80.00
	<b>Total</b>	<b>70</b>	<b>100</b>
Neonatal outcome based on gestational age at delivery	Term	34	62.96
	Moderate-late preterm	14	25.93
	Very preterm	4	7.41
	Extremely preterm	2	3.70
	<b>Total</b>	<b>54**</b>	<b>100</b>
Neonatal outcome based on neonatal birth weight	<1,500 gm	5	9.26
	1,500–2,499 gm	14	25.93
	2,500–4,000 gm	35	64.81
	<b>Total</b>	<b>54**</b>	<b>100</b>
Neonatal outcome based on Apgar score	Vigorous baby	45	83.34
	Mild asphyxia	1	1.85
	Moderate asphyxia	1	1.85
	Severe asphyxia	7	12.96
<b>Total</b>	<b>54**</b>	<b>100</b>	

\*There were nine patients who did not have chest X-ray examination

\*\*Of the 70 cases, there were 17 patients who had not given birth and 1 patient with twin pregnancy (gemelli)  
O&G, obstetric and gynecology

Table 1 shows that most of the pregnancy cases with COVID-19 in this study were in the age group 26–30-years, as much as 26 cases (37.14%) with most common comorbidities found were preeclampsia and electrolyte imbalance, as much as nine cases each (12.86%). In this study, the majority of patients were asymptomatic (30%). There were 10 cases (14.29%) with critical symptoms who were intubated and using mechanical ventilation, and 8 of them died. Fourteen cases in this study requiring intensive care, consisting of 2 cases with severe symptoms and 12 critical ill cases, and maternal death in 9 cases.

Most of the neonates born at term, with birth weight 2.500 gm and vigorous baby. There were two cases of extremely preterm birth where pregnancy termination was carried out due to worsening of the mother's condition. One patient with severe pneumonia and impending respiratory failure underwent cesarean section at gestational age 25 weeks 1 day, after receiving ventilator support for 10 days the patient's condition improved and was declared cured from COVID-19 based on clinical and negative Real time polymerase chain reaction (RT-PCR) results. One patient was terminated at gestational age 27 weeks 0 day for indications of severe acute respiratory distress syndrome and died on the fifth postoperative day.



**Table 2:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by trimester of pregnancy at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Trimester	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
First	4.25	1.96–6.17	19.2	18.23–83.7	0.03	0.02–0.03
Second	7.7	4.52–12.06	47.07	23.52–136.4	0.15	0.09–0.64
Third	6.28	0.91–20.69	35.52	0.49–197.64	0.14	0.02–42.4

**Table 3:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by maternal comorbidities at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Maternal comorbidities	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
No comorbidity	6.14	0.91–20.69	30.86	0.49–136.4	0.11	0.02–2.18
Preeclampsia	6.44	3.47–14.06	39.93	5.83–197.64	0.22	0.07–4.71
Heart failure	6.6	–	110.54	–	1.26	–
Electrolyte imbalance	6.3	1.96–17.2	56.86	19.2–151.56	0.33	0.02–42.4
Thrombocytopenia	4.66	2.57–7.45	22.78	3.00–44.6	0.17	0.06–0.34
Hepatitis B	2.73	–	3.88	–	0.05	–
Obesity	6.79	2.88–9.14	57.12	6.22–119.97	0.11	0.06–0.22

**Table 4:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by length of stay at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Length of stay	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
<10 days	4.84	0.91–17.2	24.65	0.49–197.64	0.115	0.02–42.4
≥10 days	6.93	2.57–20.69	59.83	1.60–151.56	0.145	0.03–2.56

**Table 5:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by chest X-ray feature at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Chest X-ray feature	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
Pneumonia	6.72	2.57–20.69	44.94	0.09–197.64	0.16	0.02–42.4
Without pneumonia	4.00	0.91–8.92	8.03	1.38–79.09	0.11	0.06–0.79

The highest median NLR, CRP, and procalcitonin values occurred in cases of pregnancy with COVID-19 in the second trimester in this study, 7.7 (4.52–12.06), 47.07 (23.52–136.4), and 0.15 (0.09–0.64), respectively, as shown in Table 2.

Table 3 shows the highest median NLR value was found in pregnant women with COVID-19 in comorbid with obesity, 6.79 (2.88–9.14). The highest median CRP and procalcitonin values were in patients with heart failure, 110.54 and 1.26, respectively. An increased inflammatory markers (NLR, CRP, and procalcitonin) was evaluated in obese pregnant women with COVID-19.

In this study, cases with a length of stay more than or 10 days had a median NLR value of 6.93 (2.57–20.69), CRP 59.83 (1.60–151.56), and procalcitonin 0.145 (0.03–2.56), which are all higher than those whose length of stay was less than 10 days as shown in Table 4.

Table 5 shows that it was found that each of these inflammatory markers (NLR, CRP, and procalcitonin) had a higher median value in the group of cases of pregnancy with COVID-19 with pneumonia compared to cases without pneumonia (6.72; 44.94; 0.16 vs 4.00; 8.03; 0.11, respectively).

Tables 6 and 7 shows the median values of inflammatory markers NLR, CRP, and procalcitonin were higher in patients who received oxygen supplementation compared to the group without oxygen supplementation. As the highest was evaluated in one patient with severe symptoms who used HFNC at 17.95, 105.51, and 0.16, respectively. All patients using ventilators in this study were critically ill cases.

The median value of CRP in the group requiring intensive care was higher than in the group without intensive care as shown in Table 8.<sup>41</sup>

Table 9 shows the median values of NLR, CRP, and procalcitonin in this study were highest in the group of mothers who gave birth to very preterm babies (gestational age of 28–32 weeks). Pregnancy of three women with gestational age 28–32 weeks was terminated to reduce the maternal cardiovascular and respiratory burden (iatrogenic preterm delivery) because they were in critically ill condition.

The median values of NLR, CRP, and procalcitonin were highest in the group whose neonate’s birth weight were less than 1,500 gm.

**Table 6:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by symptoms at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Symptoms	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
Asymptomatic	4.53	2.11–14.73	14.32	0.49–102.86	0.08	0.02–0.79
Mild symptoms	3.91	0.91–9.62	14.99	1.00–34.96	0.07	0.02–0.56
Moderate symptoms	4.86	2.57–9.12	39.74	5.83–91.03	0.16	0.02–2.56
Severe symptoms	8.36	6.17–17.95	105.40	44.6–145.3	0.19	0.03–1.50
Critically ill	10.31	6.1–20.69	77.93	36.4–197.64	0.47	0.08–42.4

**Table 7:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by oxygen supplementation at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Oxygen supplementation	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
No oxygen supplementation	4.37	0.91–14.73	18.96	0.49–145.3	0.115	0.02–2.56
Nasal canule	5.89	3.47–7.7	46.02	7.1–98.93	0.095	0.02–0.29
Face mask	6.79	5.48–8.09	55.0	27.54–82.46	0.13	0.1–0.16
Non-rebreathing mask	9.14	6.6–12.53	119.97	105.28–197.64	0.3	0.14–1.26
High flow nasal canule	17.95	–	105.51	–	0.16	–
Ventilator	10.96	6.1–20.69	77.93	40.07–151.56	0.47	0.08–42.4

**Table 8:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by the needs of intensive care at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Needs of intensive care	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
Intensive care	10.31	6.1–20.69	93.99	40.07–197.64	0.42	0.08–42.4
Without intensive care	5.2	0.91–14.73	28.09	0.49–145.3	0.12	0.02–2.56

**Table 9:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by neonatal outcome based on gestational age at delivery at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Gestational age at delivery	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
Term	5.14	0.91–17.95	25.19	1.00–197.64	0.115	0.02–4.71
Moderate-late preterm	6.4	2.57–12.51	39.93	0.49–151.56	0.33	0.04–2.56
Very preterm	13.16	6.3–20.69	80.31	57.2–145.3	1.04	0.08–42.4
Extremely preterm	9.16	6.25–12.06	58.89	40.07–77.71	0.39	0.15–0.63

**Table 10:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by neonatal outcome based on neonatal birth weight at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Neonatal birth weight	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
<1,500 gm	12.06	6.25–20.69	77.71	40.07–145.3	0.63	0.15–42.4
1,500–2,499 gm	7.45	3.55–17.95	44.6	1.60–151.56	0.22	0.04–2.56
2,500–4,000 gm	4.47	0.91–14.73	21.76	197.64	0.12	0.02–4.71

Four of five mothers that group were cases of critically ill COVID-19. Thus, pregnancy termination was decided at preterm gestation due to the deteriorating mother’s condition as shown in [Table 10](#).

In this study, it was found that mothers with severe asphyxia neonates had the highest median NLR and CRP values, 9.11

(6.25–20.69) and 77.71 (14.32–151.56), respectively. Meanwhile, the median value of procalcitonin was the highest in mothers with moderate asphyxia neonate which was 0.79. Of the seven mothers whose neonate had severe asphyxia, one mother had a pregnancy termination due to fetal distress and six mothers who were

**Table 11:** Overview of NLR, CRP, and procalcitonin in pregnancy with COVID-19 by neonatal outcome based on Apgar score at Sanglah General Hospital, Denpasar; Period: April 2020–April 2021

Apgar score	NLR		CRP		Procalcitonin	
	Median	Range	Median	Range	Median	Range
Vigorous baby	5.95	0.91–17.95	30.86	0.49–197.64	0.12	0.02–4.70
Mild asphyxia	3.06	–	5.68	–	0.34	–
Moderate asphyxia	8.19	–	39.93	–	0.79	–
Severe asphyxia	9.11	6.25–20.69	77.71	14.32–151.56	0.33	0.08–42.4

terminated at a gestational age ranging from extremely preterm to moderate-late preterm due to the worsening condition of the mother with COVID-19 critical ill as shown in Table 11.

## DISCUSSION

Based on maternal age, most of the cases of pregnancy with COVID-19 in this study were in the age group of 26–30 years, as much as 26 cases (37.14%). This was consistent with other studies in various countries which found that the mean maternal age of pregnant women with COVID-19 was at their reproductive age. This was similar to the mean age of non-pregnant women with COVID-19 population.<sup>3–5</sup> In this study, most pregnant women had COVID-19 during their third trimester (85.71%) which was also consistent with other studies’ results.<sup>5–7</sup> The most comorbidities found in the case of pregnancy with COVID-19 in this study were preeclampsia and electrolyte imbalance, as much as nine cases each (12.86%). Preeclampsia in pregnant women with COVID-19 may be caused by an increase in placental dysfunction due to intravascular inflammation associated with infection that causes a prothrombotic state in the blood to the placenta. Studies of the placentas of pregnant women infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) revealed a higher rate of maternal vascular malperfusion.<sup>8</sup>

In this study, the majority of patients were asymptomatic (30%). This was similar to a study conducted by Akhtar et al. which found that patients most often came with mild symptoms of infection including fever, cough, fatigue, and shortness of breath, but some were found with no symptoms (asymptomatic).<sup>9</sup> In contrast, Vakili et al. stated in their study that pregnant women are more susceptible to COVID-19 and severe pneumonia. This was due to changes in physiological adaptation and immunosuppressive conditions during pregnancy.<sup>1</sup> In this study, there were 10 cases (14.29%) with critical symptoms who were intubated and using mechanical ventilation, and 8 of them died. Several studies suggest that pregnant women have an increased risk of morbidity and an increased risk of undergoing intensive care and the use of mechanical ventilation.<sup>10</sup> A study by Khalil et al. stated that 3.4% of their study population was intubated.<sup>6</sup> Turan et al. in their study found that 51 out of 61 (83.6%) pregnant women with COVID-19 need mechanical ventilation and were treated in the ICU.<sup>11</sup> A total of 14 cases in this study requiring intensive care, consisting of 2 cases with severe symptoms and 12 critical ill cases, and maternal death in 9 cases.

Neonatal outcomes based on gestational age at delivery obtained in this study were most of the neonates born at term, having birth weight 2,500 gm, and vigorous baby. There were two cases of extremely preterm birth in this study where pregnancy termination was carried out for medical indications (indicated preterm delivery),

that is worsening of the mother’s condition. One patient with severe pneumonia and impending respiratory failure underwent cesarean section at gestational age 25 weeks 1 day, after receiving ventilator support for 10 days the patient’s condition improved and was declared cured from COVID-19 based on clinical and negative RT-PCR results. One patient was terminated at gestational age 27 weeks 0 day for indications of severe acute respiratory distress syndrome, and died on the fifth postoperative day.

In this study, it was found that the highest median NLR, CRP, and procalcitonin values occurred in cases of pregnancy with COVID-19 in the second trimester, that is 7.7 (4.52–12.06), 47.07 (23.52–136.4), and 0.15 (0.09–0.64), respectively. These were not consistent with the “immune clock” theory which states that the second trimester of pregnancy is an anti-inflammatory state in which there should be suppression of proinflammatory cytokines.

The proinflammatory state in the first and third trimesters of pregnancy is characterized by an increase in various kinds of proinflammatory cytokines. Therefore, it is very important to be aware of because pregnant women in the first and third trimesters are in a pro-inflammatory state, and the occurrence of a cytokine storm caused by SARS-CoV-2 in the first and third trimesters of pregnancy can cause a more severe inflammatory state. Thus, it will cause a more severe clinical manifestation.<sup>2</sup>

The proinflammatory state in the first and third trimesters of pregnancy is characterized by an increase in various kinds of proinflammatory cytokines. Therefore, it is very important to be aware of because pregnant women in the first and third trimesters are in a pro-inflammatory state, and the occurrence of a cytokine storm caused by SARS-CoV-2 in the first and third trimesters of pregnancy can cause a more severe inflammatory state. Thus, it will cause a more severe clinical manifestation.<sup>2</sup>

In a study conducted on 30 cases of pregnancy with COVID-19 in China, it was found that chronic disease can increase and strengthen the inflammatory response to COVID-19 compared to COVID-19 patients without a comorbid chronic disease.<sup>12</sup>

Inflammation has an important role in the progression of heart failure and is known as the “cytokine hypothesis” which states that the progression of heart failure results from the toxic effects produced by endogenous cytokine cascades in the heart and peripheral circulation. Neutrophilia is associated with an increased incidence of acute decompensated heart failure and lymphopenia is associated with a poor prognosis in patients with heart failure. Increased NLR was reported as an independent predictor of mortality and cardiovascular disease.<sup>13</sup> In the Val-HeFT (Valsartan Heart Failure Trial), it is said that CRP was a strong predictor of morbidity and mortality, and was associated with disease severity and prognosis.<sup>14</sup>

The increase in inflammatory markers due to comorbid preeclampsia can be explained that the main components

in preeclampsia are inflammation, endothelial dysfunction, angiogenesis, abnormal placentation, oxidative stress, and immunological and genetic factors. The changes in the immune system are very important in the pathogenesis of preeclampsia. In women with preeclampsia, neutrophils are activated in the placental circulation under hypoxic conditions which contribute to increased vascular resistance in preeclampsia.<sup>15</sup> Neutrophil-to-lymphocyte ratio in preeclamptic patients was significantly higher compared to those without preeclampsia.<sup>15</sup> Preeclampsia is characterized by antiangiogenetic and inflammation, thus increasing in proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), which ultimately causes the expression of the acute phase protein CRP.<sup>16</sup> In preeclampsia, there is also an increase in procalcitonin, which is associated with systemic inflammation, and increases in other proinflammatory cytokines and cytotoxic activity on hepatocytes and endothelium.<sup>17</sup>

Obese pregnant women with COVID-19 in this study had increased inflammatory markers (NLR, CRP, and procalcitonin). In a study conducted by Furuncuoğlu et al. it was found that neutrophil levels will increase along with an increase in body mass index (BMI) and there is a very significant positive correlation between BMI values and neutrophil levels ( $p = 0.000$ ).<sup>18</sup> Human adipose tissue expresses the proinflammatory cytokine IL-6, which has the potential to induce low-level systemic inflammation in overweight and obese individuals and therefore tends to have elevated CRP levels compared with those who have normal BMI. The higher the BMI, the higher the CRP concentration. These findings describe a low-grade systemic inflammatory condition in overweight and obese individuals.<sup>19</sup> Kassas et al. also concluded that procalcitonin is a significant marker of low-grade inflammation associated with obesity.<sup>20</sup>

In this study, cases with a length of stay more than or 10 days had a median NLR value of 6.93 (2.57–20.69), CRP 59.83 (1.60–151.56), and procalcitonin 0.145 (0.03–2.56) which are all higher than those whose length of stay less than 10 days. The length of stay for patients with COVID-19 was affected by the severity of symptoms, where the average length of stay in the cases of moderate symptoms and severe symptoms is 14.56 and 23.43 days, respectively.<sup>21</sup> The results of this study are similar to Erdogan et al. that found a positive correlation between NLR and length of hospital stay.<sup>22</sup> Compared with patients with a short length of stay, patients who stayed longer in the hospital had higher NLR and CRP values ( $p < 0.001$ ).<sup>23</sup>

Cytokine storm acts as a chemoattractant for neutrophils, CD4 T helper cells, and CD8 cytotoxic T cells, which then begin to sequester into lung tissue. These cells are responsible for fighting off viruses and are further responsible for inflammation and lung injury. Host cells undergo apoptosis by releasing new viral particles, which then infect adjacent type 2 alveolar epithelial cells in a similar manner. Due to persistent injury caused by sequestering inflammatory cells and viral replication leading to loss of type I and type II pneumocytes, there is diffuse alveolar damage culminating in acute respiratory distress syndrome (ARDS).<sup>24</sup>

As the infection progresses and the inflammation increases, there will be more extensive lung involvement and the chest radiograph will show bilateral multifocal alveolar opacity which may also be associated with pleural effusion.<sup>24</sup>

The chest X-ray imaging seems to positively fond with levels of CRP, LDH, and was in negative tendency with oxygen saturation. The result of chest X-ray imaging was in accordance with CT-scan imaging. The use of chest radiographs can reflect the clinical

severity of the patient.<sup>25</sup> Another study stated that CRP levels in combination with CT-scan and chest radiographs and could predict SARS-CoV-2 infection in the lungs or poor outcomes in patients with viral pneumonia.<sup>26</sup> Markowicz et al. showed that CRP levels are reliable markers of clinical and radiological severity.<sup>27</sup>

Leukocytosis and an increase in neutrophils to lymphocytes ratio were the most common findings in the group of pregnant women with laboratory and clinical confirmation of COVID-19, and an increase in CRP levels in the majority of cases of pregnancy with COVID-19.<sup>28</sup> Elevated CRP, leukocytosis, and increased NLR are reliable markers of inflammation in COVID-19 in pregnancy.<sup>1</sup>

Increased NLR is associated with clinical deterioration and death in COVID-19 patients.<sup>29</sup> Xu et al. investigated the relationship between procalcitonin, CRP, and NLR on symptoms and mortality of COVID-19 patients. This study concluded that procalcitonin and NLR levels in severe and critically ill patients were significantly higher than in patients with moderate symptoms ( $p < 0.05$ ). The CRP levels also showed a statistically increasing trend, with the lowest value in the moderate symptom group (median, 10 mg/L), then increased in the severe symptom group (26.86 mg/L), and the highest was in the critically ill patient group (median, 92.2 mg/L) ( $p < 0.05$ ).<sup>30</sup>

Neutrophil-to-lymphocyte ratio is the most significant factor in the incidence of disease severity and has a significant predictive value.<sup>31</sup> Based on a study by Liu et al., the incidence of disease with severe symptoms at age less than or 50 years and NLR  $\geq 3.13$  was 50% and at age less than 50 years and NLR  $< 3.13$  was 9.1%.<sup>31</sup> Yang et al. also found that NLR is an independent prognostic biomarker related to the progression of pneumonia in COVID-19. The NLR threshold value of 3.3 indicates a superior prognostic possibility of mild to severe clinical symptoms, with the highest sensitivity and specificity. In the group of patients with age more than or 49.5 years and NLR  $\geq 3.3$ , a total of 46.1% of patients with mild symptoms progressed to severe symptoms.<sup>32</sup> The increase in NLR is related to the severity of the disease, the level of intensive care, the need for a ventilator, and the recovery of COVID-19.<sup>32</sup> A significant positive correlation was found between procalcitonin levels and NLR in the severe symptomatic group ( $p < 0.05$ ), and indicated that an increase in NLR was followed by a gradual increase in procalcitonin values.<sup>29</sup>

The CRP is a major mediator of the acute-phase response after an inflammatory event and is primarily synthesized by IL-6-dependent hepatic biosynthesis. Induction of transcription of the CRP gene mainly occurs in hepatocytes in the liver in response to increased levels of proinflammatory cytokines, especially IL-6.<sup>33</sup> The current evidence suggests that patients with severe COVID-19 tend to have high concentrations of proinflammatory cytokines, particularly IL-6, compared to those with moderate symptoms.<sup>34</sup> A meta-analysis study also showed that CRP was associated with disease severity. COVID-19 with mild symptoms tend to have lower CRP levels.<sup>35</sup> The CRP along with D-Dimer is also the parameter that are most associated with severe pneumonia.<sup>36</sup>

Serum procalcitonin (PCT) levels increase as symptoms worsen. This suggests that PCT can be an indicator of disease severity in COVID-19 and may contribute to determining the severity of patients infected with SARS-CoV-2. Procalcitonin levels seem to depend on the severity of the disease and may be associated with bacterial coinfection.<sup>21</sup>

In this study, it was found that the median values of inflammatory markers NLR, CRP, and procalcitonin were higher in patients who received oxygen supplementation compared to the group without oxygen supplementation. This is due to the

severity of the symptoms, the inflammation that occurs will be more intense and there will be a higher increase in inflammatory markers. A study conducted by Lombardi et al. stated that high NLR and high CRP levels were showing the tendency to disease severity and mortality.<sup>37</sup> The median value of NLR, CRP, and procalcitonin in this study was found to be the highest in 1 patient with severe symptoms who used HFNC at 17.95, 105.51, and 0.16, respectively.

The low lymphocyte value at the beginning of the examination is related to the higher need for oxygen supplementation during treatment.<sup>37</sup> Another study suggests that NLR as a prognostic factor for endotracheal intubation and an independent predictor of mortality risk in COVID-19 patients.<sup>38</sup> The CRP concentrations were significantly higher in patients requiring non-invasive ventilation or intubation. The patients requiring intubation are associated with severe symptoms and have an increased neutrophil count and decreased lymphocyte count.<sup>26</sup> The CRP levels were related to the need for respiratory support such as oxygen demand ( $p = 0.001$ ) and mechanical ventilation requirements ( $p = 0.004$ ). Research conducted by Markowicz et al. found a CRP cut-off value of 100 mg/L, and above this value, all patients required oxygen therapy.<sup>27</sup> In a cohort study of 93 COVID-19 patients who were mechanically ventilated, an association was found between procalcitonin levels less than 0.1 ng/mL and the risk of prolonged mechanical ventilation.<sup>39</sup>

A study conducted by Eke et al. found that an increase in NLR was an independent risk factor for the need for ICU care. The study obtained a cut-off value of  $NLR > 5.3$  (72.73% sensitivity and 91.11% specificity) in predicting the need for ICU care and concluded that NLR in COVID-19 patients is a cheap, fast, easy, and very reliable test in determining the severity of SARS-CoV-2 infection and predicting the need for ICU care.<sup>40</sup> The severity of inflammation and the severity of symptoms were in positive tendency with high CRP values. This is consistent with the results of this study which found that the median value of CRP in the group requiring intensive care was higher than in the group without intensive care.<sup>41</sup>

Based on the gestational age at delivery, the median values of NLR, CRP, and procalcitonin in this study were highest in the group of mothers who gave birth to very preterm babies (gestational age of 28–32 weeks). This was because these inflammatory markers were in positive tendency with the severity of COVID-19 symptoms. Overproduction of proinflammatory cytokines (cytokine storm) in pregnant women with COVID-19 is associated with abnormal pregnancy outcomes, such as preterm labor, preterm premature rupture of membranes, and chorioamnionitis.<sup>42</sup>

There were three pregnant women with gestational age 28 COVID-19 32 weeks in this study who had their pregnancy terminated for medical indications to reduce the maternal cardiovascular and respiratory burden (iatrogenic preterm delivery) because they were in critically ill condition. This was consistent with a systematic review done by Papapanou et al. which found an increase in the frequency of cesarean sections and the rate of preterm birth due to iatrogenic reasons. Turan et al., also found that 49.6% of preterm deliveries occurred with the primary indication of maternal SARS-CoV-2 infection rather than fetal distress. The main indication for preterm delivery is related to severe maternal pneumonia due to COVID-19 or fear of sudden worsening of maternal condition.<sup>11</sup>

In this study, it was found that mothers with severe asphyxia neonates had the highest median NLR and CRP values, 9.11 (6.25 COVID-19 20.69) and 77.71 (14.32 COVID-19 151.56), respectively.

Meanwhile, the median value of procalcitonin was highest in mothers with moderate asphyxia neonate which is 0.79. Of the seven mothers whose neonate had severe asphyxia, one mother had a pregnancy termination due to fetal distress and six mothers who were terminated at a gestational age ranging from extremely preterm to moderate-late preterm due to the worsening condition of the mother with COVID-19 critical ill. A higher neonatal morbidity, a higher is the need for NICU care, and a higher incidence of preterm birth were found in pregnant women with severe or critically ill COVID-19. This showed that both hypoxemia and respiratory failure in pregnant women with severe or critically ill COVID-19 can cause pre-placental hypoxia which is leading to fetal distress, preterm delivery, and stillbirth.<sup>11</sup>

## CONCLUSION

The descriptions of NLR, CRP, and procalcitonin of pregnant women with COVID-19 at Sanglah General Hospital, Denpasar, Bali, Indonesia for the period April 2020 moderate-late April 2021 were as follows: the highest median NLR, CRP, and procalcitonin values were in the second trimester; the highest median NLR value was in maternal with comorbid obesity, while the highest median value for CRP and procalcitonin were in maternal with comorbid heart failure; the median NLR, CRP, and procalcitonin values were higher in the group with more than or 10 days of hospitalization; the median values of NLR, CRP, and procalcitonin were higher in the group with pneumonia on their chest X-ray result; the median NLR and procalcitonin values were highest in the critically ill group, while the median CRP values were highest in the group with severe symptoms; the highest median NLR value is in the group who used high flow nasal canule as their oxygen supplementation, while the highest median CRP value is in the group who used non-rebreathing mask as their oxygen supplementation, and the highest median procalcitonin value is in the ventilator user group; the median NLR, CRP, and procalcitonin values were higher in the groups requiring intensive care. For the neonatal outcome the median values of NLR, CRP, and procalcitonin were highest in the group who gave birth at very preterm gestational age and neonate's birth weight less than 1,500 gm; and the highest median NLR and CRP values in the mother who gave birth severe asphyxia neonates, while the highest median value of procalcitonin was in mother who gave birth moderate asphyxia neonates.

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