Paediatrica Indonesiana

VOLUME 56 January • 2016 NUMBER 1

Original Article

Serum interleukin-6 and mean platelet volume in pediatric pneumonia

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Abstract

Background In pneumonia, interleukin (IL)-6 is released in response to inflammation. Interleukin-6 stimulates megakaryocyte maturation, leading to larger platelets being released into the circulation. Platelet size is measured as mean platelet volume (MPV). The MPV may also be affected by nutritional status and smoking.

Objective To assess for a possible relationship between serum IL-6 concentration and MPV, including smoking and nutritional status as confounding factors, in children with pneumonia.

Methods An analytic, observational study with cross-sectional design and consecutive sampling of children aged 2 to 59 months with a clinical diagnosis of pneumonia was conducted from November 2013 to March 2014 in Dr. Hasan Sadikin General Hospital and two network hospitals. All patients underwent routine complete blood counts including MPV and measurement of serum IL-6 concentration using an *enzyme-linked immunosorbent assay* (ELISA) technique. Regression linear analysis was used to assess the relationship between MPV and IL-6, passive smoking, and nutritional status.

Results There were 67 patients enrolled in the study. Subjects' mean serum IL-6 concentration was 49.3 (SD 78.3) pg/mL, and mean MPV was 9.2 (SD 0.9) fL. The regression model for MPV was 7.531 + 0.662 (passive smoking) + 0.276 (weight per age) + 0.009 (IL-6).

Conclusion There was a relationship between IL-6 serum concentration and MPV in children with pneumonia. **[Paediatr Indones. 2016;56:57-61.]**

Keywords: *pneumonia, interleukin-6, mean platelet volume, children*

Pneumonia is the leading, global cause of death in children under 5 years of age. More than 80% of deaths occur in low- and middle-income countries, and more than half of the deaths occur in just 6 countries: India, China, Pakistan, Bangladesh, Nigeria, and Indonesia.[1] According to the 2012 Indonesian Health and Demographic Survey, pneumonia is the leading cause of mortality in children under 5 years of age in Indonesia.[2] Pneumonia can be broadly defined as inflammation of lung tissue caused by an infectious agent that stimulates a response resulting in damaged lung tissue.[3] In pneumonia, pro- and anti-inflammatory cytokines are released in response to inflammation. In severe pneumonia, the cytokine response is not only found in the affected lung, but also in the circulation. In pneumonia patients, increased cytokines in the circulation are related to disease severity,[4,5] and higher cytokine release into the circulation is indicative of a higher the risk of mortality. Previous studies have shown that only interleukin (IL)-6, among many

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cytokines, has a major role in pneumonia, is related to disease severity, and can be used as an indicator of outcome.[6-8] In an animal trial, IL-6 was shown to affect megakaryocytopoiesis, leading to bigger platelets released into the circulation.[9] Platelet size can be measured as mean platelet volume (MPV), an index of platelet volume.[10] In addition, other factors affect MPV, such as active and passive smoking as well as nutritional status, as shown in a Korean study.[11]

The aim of this study was to assess for a relationship between serum IL-6 concentration and MPV in children with pneumonia, taking into account several other factors that may affect MPV.

Methods

This was an analytic, observational study with cross-sectional design and consecutive sampling in children aged 2 to 59 months with clinical diagnoses of pneumonia. This study was conducted from November 2013 to March 2014 in Dr. Hasan Sadikin General Hospital, Cibabat General Hospital, and Astana Anyar Mother and Child Hospital. We excluded patients with concurrent cyanotic congenital heart disease, renal failure, or acute post-streptococcal glomerulonephritis. The minimum required sample size was calculated to be 67. Informed consent was obtained from subjects' parents. All subjects underwent history-taking, physical examination, and anthropometric data measurement at the Emergency Unit. Classification of pneumonia severity was made in the Emergency Unit by pediatric residents. Blood specimens were taken in two tubes: one tube containing 2 mL of blood for measurement of routine complete blood count including MPV, and a second tube containing 3 mL of blood for measurement of IL-6. All blood specimens were sent to the Clinical Pathology Laboratory at Dr. Hasan Sadikin General Hospital within 30 minutes of the blood draw. The second tube was centrifuged in 3,000 rpm for 5 minutes, with the resulting serum layer stored at -20^{o}C. Measurement of serum IL-6 concentration using an enzyme-linked immunosorbent assay (ELISA) technique was done later after specimens had been collected from all subjects.

The independent variable was serum IL-6, the dependent variable was MPV, and confounding variables were age, passive smoking, and nutritional status. Pneumonia was defined as a clinical condition characterized by fever, tachypnea, chest retraction, crackles on thoracic auscultation, and infiltrate observed in the chest X-ray. The severity of pneumonia was categorized as mild and severe based on *The British Thoracic Society* criterias (**Table 1**).[12] Interleukin-6 was a 26 kD glycoprotein, measured as pg/mL. Mean platelet volume was an index of platelets which reflects size, measured as fL. Underweight was defined to be below a *z*-score line of -2, and severely underweight was defined to be below a *z*-score line of -3, for weight/age. Stunted was defined to be below a *z*-score line of -2, and severely stunted was defined to be below a *z*-score line of -3, for length or height/age. Wasted was defined to be below a *z*-score line of -2, and severely wasted was defined to be below a *z*-score line of -3, for weight/length or height.

Statistical analysis was conducted with *SPSS version 15.0* software. The correlation between two numerical variables was analyzed by Pearson's correlation, while categorical and numerical variables were analyzed by Spearman's correlation. Regression model was used to further analyze relationships among variables with P values under 0.25 in the bivariate analysis. The formula used was y=Bx+E (y for dependent variable, i.e., MPV; B for regression coefficient; and E for other variables). The study protocol was approved by the Ethics Committee of Medical Research, Padjadjaran University Medical School/Hasan Sadikin Hospital, Bandung, Indonesia.

Results

The general characteristics of the 67 subjects is shown in **Table 2**. Bivariate analysis results of the possible associations between MPV and serum IL-6 concentration, nutritional status, and passive smoking are shown in **Table 3**. Linear regression multivariate analysis with MPV as the dependent variable and passive smoking, weight per age, and IL-6 as independent variables, is shown in **Table 4**. The linear regression model was MPV = 7.531 + 0.662 (passive smoker) + 0.276 (weight per age) + 0.009 (IL-6), with R^2 of the model was 0.764.

Table 1. Assesment of pneumonia severity

	Mild pneumonia	Severe pneumonia
Infants	Temperature < 38.5ºC Respiratory rate <50 breaths/min Mild recession Taking full feeds	Temperature > 38.5ºC Respiratory rate > 50 breaths/min Moderate to severe recession Nasal flaring Cyanosis Intermittent apnea Grunting respiration Not feeding Tachycardia* Capillary refill time ≥ 2 s
Older children	Temperature < 38.5ºC Respiratory rate < 50 breaths/min Mild breathlessness No vomitting	Temperature > 38.5ºC Respiratory rate > 50 breaths/min Severe difficulty in breathing Nasal flaring Cyanosis Grunting respiration Signs of dehydration Tachycardia* Capillary refill time ≥ 2 s

*values to define tachycardia vary with age and with temperature

Table 2. General characteristics of subjects

Characteristics	(N=67)
Male gender, n (%)	40 (60)
Mean age (SD), months	9.2 (8.2)
Passive smoking, n (%)	63 (94)
Pneumonia severity, n (%)	
Mild	30 (45)
Severe	37 (55)
Nutritional status, n (%)	
Underweight	18 (27)
Severely underweight	17 (25)
Stunted	13 (19)
Severely stunted	10 (15)
Wasted	8 (12)
Severely wasted	2 (3)
Mean serum IL-6 concentration (SD), pg/mL	49.3 (78.4)
Mean MPV (SD), fL	9.2 (0.9)
Mean hemoglobin level (SD), g/dL	10.5 (1.8)
Mean hematocrit (SD), %	31 (5.1)
Mean leukocyte count (SD), /mm^3	13,652.1 (9,384)
Mean platelet count (SD), /mm^3	301,889.5 (180,011.5)

SD=standard deviation

Table 3. Results of association between MPV and some variables

Variables	Association with MPV
IL-6	r= 0.84* P< 0.001
Passive smoking	r= 0.22** P= 0.022
Weight per age	r= 0.49** P< 0.001
Length or height per age	r= 0.34** P= 0.004
Weight per length or height	r= 0.22** P= 0.62

*Pearson's; **Spearman's correlation

Discussion

In our study, pneumonia mostly occurred in children of a younger age, with passive smoking, and anemia as risk factors. We also observed elevated serum IL-6 concentration in pneumonia. Although abnormal levels of several cytokines, such as TNF-α, IL-1, IL-6, IL-8, and IL-10, may be observed in pneumonia patients,[5-8] only IL-6 has a major role related to disease severity.[6,8]

An effect of IL-6 on platelets has been observed.[9,13,14] Physiologically, the rate of platelet production normally increases in response to thrombocytopenia and decreases in thrombocytosis. Platelets are derived from the cytoplasm of megakaryocytes. Megakaryocytes are derived from multipotent stem cells which develop to megakaryocyte progenitor cells. Hematopoietic growth factors and cytokines affect megakaryocytopoiesis in different sites, act as stimulators or inhibitors, and increase platelet numbers or size. Interleukin-6 affects the maturation of megakaryocytopoiesis, but not production.[13]

Table 4. Linear regression analysis

Dependent variable	Independent variables	Coefficient	Coefficient correlation	P value
MPV	Constant	7.531		<0.001
	Passive smoking	0.662	0.175	0.006
	Weight per age	0.276	0.250	<0.001
	IL-6	0.009	0.775	<0.001

An effect of IL-6 on platelet size has been shown in animal trials.[9,14] Mean platelet value is known to increase in acute myocardial infarction, ischemic heart disease, ischemic stroke, gestational diabetes, pregnancy with glucose intolerance, pediatric acute post-streptococcal glomerulonephritis, and cyanotic heart disease.[11,15] Decreased MPV values occur in pulmonary tuberculosis.[16]

The mean MPV value of our subjects was higher than that of a previous study, which reported a mean MPV value in pneumonia of 7.1 (SD 0.6) fL.[17] This difference may have been due to differing criteria for diagnosing pneumonia or the design of the study.

The mechanism of action of IL-6 on platelet size in pneumonia patients is unclear. The cell cycle comprises two phases: interphase and mitosis. Interphase starts with the G1 phase, a period in which cells increase in mass. In this phase, proteins and other cellular molecules are synthesized, but DNA replication does not occur. Subsequently, S phase occurs, during which all nuclear DNA is duplicated. The end of interphase is known as the G2 phase, during which proteins are synthesized to prepare cells to enter mitosis. After completing mitosis and duplication, cells enter the next interphase period. Most variations of elongation of time in each phase occur in G1 phase.[18] Interleukin-6 is assumed to affect the cell cycle through the elongation of G1 and or G2 phase.

A Korean study observed MPV values in children with several conditions, and found that normal MPV values were 8.21 (SD 0.65) fL in neonates, and 7.60 (SD 1.26) fL in infants above 1 month, up to 48 years of age. This study also showed that MPV was related to smoking and nutritional status, but without an explanation of the mechanism.[11] In our study, all subjects' MPV values were higher than normal values. We also found relationships between passive smoking and weight per age, as part of nutritional status, and MPV value.

Cigarette smoke is assumed to affect megakaryocytopoiesis through several inflammatory cytokines. Cigarette smoke induces a significant recruitment of neutrophils in the bronchoalveolar space and pulmonary parenchyma, leading to production of IL-1, IL-6 and TNF-α, which are crucial for cigarette smoke-induced inflammation.[19] Nutritional status is assumed to affect megakaryocytopoiesis by inflammation and the influence of cytokines. A previous study showed that weight loss induced a significant reduction in IL-6.[20] In contrast, higher weight per age was associated with increased MPV in our study.

A limitation of our study was that other factors that influence megakaryocytopoiesis such as thrombopoietin and hematopoietic factors, were not included in our analysis. But to the best of our knowledge, this is the first study to describe an association between serum IL-6 concentration and MPV in children with pneumonia, including factors that might affect MPV values, such as passive smoking and nutritional status.

In conclusion, there is a relationship between serum IL-6 concentration and MPV in children with pneumonia.

Conflict of interest

None declared.

References

1. WHO/UNICEF. Global action plan for prevention and control of pneumonia (GAPP). World Health Organization/ The United Nations Children's Fund. 2009. p. 1-18.
2. Balai Pusat Statistik. Survei demografi dan kesehatan Indonesia 2012. Jakarta: Balai Pusat Statistik; 2012. p. 108-116.
3. Marostica PJ, Stein RT. Community-acquired bacterial pneumonia. In: Wilmott RW, Boat TF, Bush A, Chernick V,

Deterding RR, Ratjen F, editors. Disorder of the respiratory tract in children. 8th ed. Philadelphia: Elsevier Saunders; 2012. p. 461-72.

4. Martinez R, Menendez R, Reyes S, Polverino E, Cilloniz C, Martinez A, *et al*. Factors associated with inflammatory cytokine patterns in community-acquired pneumonia. Eur Respir J. 2011;37:393-9.
5. Endeman H, Meijvis SC, Rijkers GT, van Velzen-Blad H, van Moorsel CH, Grutters J C, *et al*. Systemic cytokine response in patients with community-acquired pneumonia. Eur Respir J. 2011;37:1431-8.
6. Antunes G, Evans SA, Lordan JL, Frew AJ. Systemic cytokine levels in community-acquired pneumonia and their association with disease severity. Eur Respir J. 2002;20:990-5.
7. Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR, *et al*. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med. 2007;167:1655-63.
8. Michelow IC, Katz K, McCracken GH, Hardy RD. Systemic cytokine profile in children with community-acquired pneumonia. Pediatr Pulmonol. 2007;42:640-5.
9. Burstein SA, Downs T, Friese P, Lynam S, Anderson S, Henthorn J, *et al.* Thrombocytopoiesis in normal and sublethally irradiated dogs: response to human interleukin-6. Blood. 1992;80:420-8.
10. Giovanetti TV, do Nascimento AJ, de Paula JP. Platelet indices: laboratory and clinical applications. Rev Bras Hematol Hemoter. 2011;33:164-5.
11. Kim KY, Kim KE, Kim KH. Mean platelet volume in the normal state and in various clinical disorders. Yonsei Med J. 1986;27:219-26.
12. Harris M,Clark J,Coote N,Fletcher P, Harnden A. British Thoracic Society guidelines for the management of community acquired pneumonia in children: update 2011. Thorax. 2011;66:1-23.
13. Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. J Interferon Cytokine Res. 2002;22:913-22.
14. Stahl CP, Zucker-Franklin D, Evatt BL, Winton EF. Effects of human interleukin-6 on megakaryocyte development and thrombocytopoiesis in primates. Blood. 1991;78:1467-75.
15. Slavka G, Perkmann T, Haslacher H, Greisenegger S, Marsik C, Wagner OF, *et al*. Mean platelet volume may represent a predictive parameter for overall vascular mortality and ischemic heart disease. Arterioscler Thromb Vasc Biol. 2011;31:1215-8.
16. Gunluoglu G, Yazar EE, Veske NS, Seyhan EC, Altin S. Mean platelet volume as an inflammation marker in active pulmonary tuberculosis. Multidiscip Respir Med. 2014;9:11.
17. Karadag-Oncel E, Ozsurecki Y, Kara A, Karahan S, Cengiz AB, Ceyhan M. The value of mean platelet volume in the determination of community acquired pneumionia in children. Ital J Pediatr. 2013;39:16.
18. Pollard T, Earnshaw W. Cell biology. Philadelphia: Elsevier; 2008. PAGES?
19. Doz E, Noulin N, Boichot E, Guénon I, Fick L, Le Bert M, *et al*. Cigarette smoke-induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling dependent. J Immunol. 2008;180:1169-78.
20. Kopp HP, Kopp CW, Festa A, Kryzanowska K, Kriwanek S, Minar E, *et al*. Impact of weight loss on inflammatory proteins and their asociation with the insulin resistance syndrome in morbidly obese patient. Arterioscler Thromb Vasc Biol. 2003;23:1042-7.