INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 300
2023 July; 29(3): 300 - 305
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Hematology Reference Values in Indonesian Children

Ina S. Timan[1,2], Aryati[3]

[1] Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. E-mail: ina_st_ui@yahoo.com
[2] Faculty of Medicine and Health Sciences, Universitas Kristen Krida Wacana, Jakarta, Indonesia
[3] Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

ABSTRACT

Every clinical laboratory has to establish its reference ranges for every parameter analyzed. These reference values are intervals considered normal in a healthy person's physiological condition. It will be used by the clinician or other health professionals to interpret the laboratory test results of the patient for making diagnostic decisions, monitoring patient therapy, and predicting the prognosis and it is also used in epidemiology studies. A reference range is defined as an interval in which 95% of a reference population's values fall. It is very crucial to establish reference intervals for the local population as it is sometimes affected by ethnicity, nutrition, food habits, and economic and other local conditions. Hematology is the most common test performed in a clinical laboratory, and the most used parameter by the clinician including pediatricians. There up to now no well-established reference interval for hematological parameters in pediatrics and children in Indonesia. The reference interval used for hematology parameters in Indonesia is based on reference intervals from other countries or the manufacturer of the hematology analyzers used in a particular laboratory. Establishing a population-specific hematology reference interval is very difficult for pediatrics and children, so it is rarely performed. This study aims to compile the results of hematology parameters from Indonesian children to establish an Indonesian reference value for hematology. The method used was compiling data for the hematology parameter intervals based on studies performed in the Indonesian population and presented as 95% confidence intervals. A pediatric reference range for several hematology parameters has been established based on 3 groups of age in Indonesian children.

Keyword: Hematology reference interval, Indonesian children

INTRODUCTION

Hematology parameters are the most common test asked by clinicians, especially pediatricians for making clinical decisions. Hematology parameters are mostly performed using automated blood cell counters, in Indonesia the parameters analyzed are for hemoglobin, hematocrit, red blood cell count, red blood cell indices, white blood cell count, white blood cell differential count, and platelets. These are the most basic hematology tests performed in a basic small blood cell counter. Many larger instruments can perform more than 30 hematology parameters simultaneously using only around 150-300 mL whole blood. It is a very efficient test, using a small amount of blood, and generating many useful parameters, especially in babies and small children.[1,2]

Hematology parameters give information for use in routine assessment for diagnosis of red blood cells, white blood cells, platelet disorders, infectious diseases, immune status, choosing the right management plan for the patients, monitoring the outcome of therapy, and predicting the prognosis. An age-related hematology reference value is needed for the interpretation of the results. Hematology parameters are often used as a screening test and based on the results in comparison with the reference values, the clinician can order other parameters for diagnosing the condition of the patients.[2,3]

Reference ranges can differ based on gender, age, geographic condition, altitude, ethnic background, nutrition, and food habit in each country. Normal hematology values presented as the reference value is needed for every country for optimal care of the patients. The reference range for pediatric patients are very difficult to obtain, unlike adult reference values, which can be obtained from medical check-up patients or blood donors, healthy baby or children are rarely asked to be tested. No current Indonesian-based pediatric hematology reference values are available, hopefully, this reference value will be very useful for the care of the children in Indonesia.[1,4,5] By compiling data from several studies in Indonesia, a more specific reference range for Indonesian children can be established.

METHODS

By compiling data from reports and publications on the hematology parameter studies in Indonesia a reference range was established. Data were compiled from a study of 1-year-old healthy babies, healthy primary school children in Jakarta, and secondary school children in West Java. All of these studies were performed in Cipto Mangunkusumo Hospital using Sysmex automated blood cell counters. The reference values were categorized into 3 groups; babies, children from 8 to 12 years old, and children older than 12 to 15 years old.[6-9]

Results for reference values of hematology in children

The reference range for hematology parameters in various age groups is different. The reference values interval in hematology is usually categorized as newborns, infants, babies, children, and teenagers or young adults.[1,5,6] In Table 1 the hematology parameters are grouped into babies (1 year old), primary school-aged children (between 7 to 12 years old), and secondary school-aged teenagers (between 13 to 15 years old). Results were quoted from 3 different studies, all subjects were healthy children, as evaluated by pediatricians. The studies were performed in Jakarta and West Java, Indonesia, all using Sysmex hematology analyzers.[7-9]

The results of the hematology parameters in 12 months old babies were deducted from studies in Jakarta, in middle-income families. The reference values in primary school-age children aged 8-12 years old were taken from studies in Jakarta, also in middle-income families, and the reference values in the older children were from secondary school

Table 1. The reference value of hematology parameters in babies, children and teenagers[7-9]

Parameter	Unit	Mean	SD	Range
Baby 12 months (N=100)				
Hemoglobin	g/dL	11.5	0.99	9.5-13.5
Hematocrit	%	34.8	2.56	30-40
Red Blood Cell (RBC)	$10^6/\mu L$	4.69	0.32	4.05–5.33
Mean Cell Volume (MCV)	fL	74.4	4.81	65-84
Mean Cell Hemoglobin (MCH)*	pg	24.9		20-28
Mean Cell Hemoglobin Concentration (MCHC)	g/dL	33.1	1.26	31-36
White Blood Cell (WBC)*	$10^3/\mu L$	12.6		7.4–17.5
Platelet *	$10^3/\mu L$	267	155.09	106–523
Red Cell Distribution Width (RDW-SD)	fL	40.9	3.31	34.5-47.1
Children 8-12 years (N=120)				
Hemoglobin (Hgb)	g/dL	12.8	0.86	11.1–14.5
Hematocrit (Hct)	%	36.3	2.11	32.1–40.5
Red Blood Cell (RBC)	$10^6/\mu L$	4.55	0.29	3.97–5.13
Mean Cell Volume (MCV)	fL	80	3.37	73–87
Mean Cell Hemoglobin (MCH)*	pg	28.1		26-30
Mean Cell Hemoglobin Concentration (MCHC)	g/dL	35.2	0.91	33-37
White Blood Cell (WBC)*	$10^3/\mu L$	8.2	1.97	4.3–12.1
Platelet (PLT)*	10^3/mL	369		204_534
Children-Teenagers 12-15 years (N= 60)				
Hemoglobin (Hgb)*	g/dL	13.3		11.2-15.2
Hematocrit (Hct)*	%	39.4		34–43.3
Red Blood Cell (RBC)	$10^6/\mu L$	4.77	0.47	3.83–5.71
Mean Cell Volume (MCV)*	fL	83.4		61.4-89.6
Mean Cell Hemoglobin (MCH)*	pg	28.3		19.3–30.4
Mean Cell Hemoglobin Concentration (MCHC)*	g/dL	33.9		32-35
White Blood Cell (WBC)	$10^3/\mu L$	7.5	1.64	4.2–10.8
Platelet (PLT)	$10^3/\mu L$	311	69.08	173-449
Red Cell Distribution Width (RDW-CV)*	fL	13.1		11.8–16.6

Results were calculated as mean ±2SD except those marked with * were calculated as 5th to 95th percentile

children in West Java, also in middle-income families.[7-9]

The use of reference values

Reference interval values should be provided for each laboratory result produced by the laboratory. It is used as a decision-making tool to differentiate between the healthy and diseased populations, for physicians to determine the management of the patients. Reference values are usually derived from normal healthy subjects. Determination of reference range values is very difficult in pediatric subjects; it changes with age due to physiological developments from birth to adolescence and the rare opportunity to get blood from healthy pediatric subjects. Normal subjects can also give different results based on the nutrient variation, socio-economic condition, and different altitudes in each area of Indonesia.[10,11]

Hematology parameters basically consisted of the parameters for RBC, WBC, and platelets. Each group consisted of several parameters. Modern blood cell counters can give more extended results based on calculation and conventional impedance method, they can also use flow cytometry and cytochemistry for detection and calculations.[1]

It is sometimes difficult to collect venous blood from babies and very small children. A study to evaluate the measurement of CBC by venous and capillary blood in children below age 5-9 years with acute gastroenteritis showed that it has still good reliability with sensitivity and specificity of 86-98% and accuracy of 95%. Comparison of matched pairs showed some slightly higher red blood cell counts, hemoglobin, and platelets.[12]

Detection of anemia and polycythemia

Containing oxygen-carrying capacity, causing insufficiency to meet the body' Hematology parameters are very useful for clinicians. It gives information on the condition and changes in RBC, WBC, platelets, and their cross interactions. The RBC parameters can give information on the hemoglobin, hematocrit, RBC count, RBC indices, and red cell distribution width. Based on the results clinicians can interpret whether the subject is normal, have anemia or hemoconcentration, or polycythemia. Anemia is defined as a deficiency in the concentration of RBC and the hemoglobin s physiological needs. It can also give information on the type of anemia, suspected nutrient deficiency, hemoglobinopathies, and other red cells abnormalities.[1,4,13]

The red cell indices, such as MCV are used for quantifying the size of the RBC and classifying anemia as normocytic, microcytic, and macrocytic. MCH is the average amount of hemoglobin in RBC, its value lowers in iron deficiency and other hypochromic anemia. MCHC is the average concentration in a certain volume of RBC, the ratio of hemoglobin to hematocrit. The MCH and MCHC are used to measure the hemoglobin concentration in the RBC, classifying it as hypochromic, normochromic, or hyperchromic. The RDW can give information on normal or increased variation in the size of RBC, known as the degree of anisocytosis. MCHC can be used together with MCV and RDW for determining the possible cause of anemia. Normal MCHC values can be observed in kidney disease, bone marrow failure, and hemolytic anemia. Low MCHC can be found in iron deficiency, thalassemia, sideroblastic anemia, and anemia of chronic disease. An increase in MCHC can occur in hereditary spherocytosis and liver disease.[14]

An increase in RDW even within normal limits of normal CBC results, should be confirmed by blood smear and further investigated. RDW changes can be observed in impending anemia, iron deficiency, hemoglobinopathies, and kidney disease. The combination of the anemia type and RDW will be more useful. Normal RDW with microcytic MCV can be found in thalassemia, normal RDW with normocytic MCV can be observed in anemia of chronic disease and hereditary spherocytosis, an RBC with macrocytic MCV can be found in liver disease or preleukemic condition. High RDW in microcytic MCV can be observed in iron deficiency anemia, and high RDW with normocytic RBC can be found in combined anemia, sickle cell disease, and sideroblastic anemia. High RDW with macrocytic red blood cells can be found in B12 and folate deficiency.[14,15]

Results of red cell abnormalities can be used as a base to do more specific tests to know the cause of the abnormalities. Pediatric anemia can be caused by acute, chronic, or iatrogenic blood loss; decreased erythrocyte production; increased destruction of erythrocytes, as with hemolysis; or shortened erythrocyte survival. Polycythemia is most commonly defined as a venous hematocrit greater than 65%.[1,4,13]

Reference values and cut-off points from publications can identify populations with a higher risk of anemia and facilitate its management and monitoring, also assessing prevention methods.[5] The World Health Organization (WHO) has recommended the classification of anemia based on hemoglobin levels (Table 2).[4,15]

The WHO cut-off points for determining anemia in babies and children differed. The lower

Table 2. Anemia classification by WHO[4]

Age Population	Hemoglobin (g/dL)			
	Non-Anemia	Mild Anemia	Moderate Anemia	Severe Anemia
6-50 months	≥ 11.0	10-10.9	7- 9.9	< 7.0
5-11 years	≥ 11.5	11-11.4	8-10.9	< 8.0
12-14 years	≥ 12.0	11-11.9	8-10.9	< 8.0

hemoglobin reference values for babies, children, and teenagers in Indonesia were slightly lower from 13.6%, 3.5%, and 6.7%, respectively in each age group. This should be considered when hemoglobin levels are used for determining anemia in epidemiological studies or in clinical practice. The reference values for hemoglobin, hematocrit, and RBC indices (MCV and MCH) showed a slight increase by age group.[7-9]

The value of RDW can also be applied for screening iron deficiency anemia and differentiating it from thalassemia.[16] Red cell distribution width can be expressed as RDW-SD or RDW-CV, with the normal reference of 42.5±3.5 fL and 12.8±1.2%, respectively. These values are almost reported the same in children and adults. The values of RDW reported in the Indonesian children study is almost the same (Table 1).[1,7-9]

Nutritional-related anemia is still one of the major burdens in the health care system in the world including Indonesia, most subjects are treated as iron deficient anemia. Through several studies, parameters used to detect the anemia were the measurement of hemoglobin and hematocrit, some also included the reticulocyte hemoglobin measurement as a monitoring test. In a study based on Riskesdas 2018, it was reported that 40.4% of children under 5 years old in Indonesia had anemia with 11.2% moderate and severe anemia. Anemia was diagnosed if the hemoglobin levels were below 11 g/dL. Most anemia was reported only based on hemoglobin levels, and erythrocyte indices, which could predict the cause of anemia were not usually analyzed. It can be observed many presumed healthy babies have hemoglobin levels under 11 g/dL. Although to diagnose iron deficiency other tests should be done, such as serum iron, total iron binding capacity, and ferritin measurement, most anemia in Indonesia were presumed as iron deficiency. Iron supplements are rarely given to children below 12 years old. Other nutritional anemia was very rarely reported although intervention given usually included folic acid and other vitamins.[17,18]

Reference values for hematology are a progressive condition based on age. In a study in Korea in 66.611 children aged 3-17 years old it was found that RBC increased with age until adulthood, and decreased in females after puberty. There were no differences according to gender in children before puberty.[19]

White blood cells and platelet abnormalities

Using the reference value for WBC or leukocytes, clinicians can suspect or confirm the presence of infection, inflammation, or other conditions. White blood cells are the main body defense against invading microorganisms. The WBC is usually differentiated into granulocytes and non-granulocytes. More sophisticated hematology analyzers can perform a 5 or 7 differential count in WBC count using flow cytometry-based tests.[1] Increased WBC or leukocytosis can be found in most bacterial infections, but some infections can cause leucopenia. A very high count of WBC can also be found in leukemoid reactions or leukemia, which should be confirmed by morphology studies or other tests. Low WBC count can be found in viral infection, sepsis, or other abnormalities in the marrow. As observed in Table 1, the WBC slightly decreased in older children groups. White blood cell count can be used for monitoring after treatment of inflammation, infection and certain malignancy. White blood cell count can differ up to 14% when taken in the morning in resting subjects, compared to other times of the day, although the diurnal variation is not clear. The WBC count in the babies and children age group is within the same range reported, babies 11±5 10^3/mL, children 9±4 10^3/mL after 12 years old 4-10 10^3/mL.[1,20]

The total WBC count and monocyte count were highest at birth then rapidly decreased in the first 6 months of life, then showed a slight reduction until 2 years of age. Lymphocyte counts were low during infancy and increased to their highest level at 6 months of age, decreasing moderately again until 9 years of age. The pattern of neutrophil count changed in the opposite of the lymphocyte. There were no patterns of eosinophil and basophil that were related to changes in age. Gender differences were only observed after puberty. Physiological development had some effect on the changes in the hematology parameters during infancy to puberty.[21]

Changes in the platelet count can be identified as thrombocytosis (increase in platelet count) or thrombocytopenia (decrease in platelet count). Changes in platelets can be caused by a decrease in

production, sequestration, trapped in the spleen, or due to small platelet clumps. These conditions can be observed in bacterial or viral infection, sepsis, disseminated intravascular coagulation, autoimmune diseases, congenital anomaly syndromes, bleeding, and other conditions. Thrombocytosis can also be observed as a physiological reaction in many conditions, or due to contamination of small RBC. Thrombocytosis can be related to a reactive condition such as chronic infection and inflammation, malignancy, post-splenectomy, acute blood loss, and iron deficiency. It can also be related to myeloproliferative neoplasm (essential thrombocytosis, polycythemia vera) or as a part of a congenital disorder.[14]

Thrombocytopenia can also be found due to small platelet aggregations in autoimmune conditions or in reactive platelets. Thrombocytopenia is often observed in viral infections such as dengue hemorrhagic syndrome, COVID-19, post-chemotherapy, congenital disorders (Gaucher disease, neonatal autoimmune thrombocytopenia), splenomegaly or after transfusion, Disseminated Intravascular Coagulation (DIC).[14] Low platelet count should be confirmed to ensure that it was not caused by a technical error, check the integrity of the sample, check for the presence of microclots, and check in the blood smear for platelet satellitism and clumping. Platelets showed a slight diurnal variation of up to 5%, megakaryocyte platelet release mostly happens late at night and early morning. The platelets showed a slight decrease in the increasing age groups. Platelet count reported in the Indonesian children is within the same range, babies 200-550 10^3/mL, children 170-450 10^3/mL after 12 years old 280±180 10^3/mL.[1]

Hematology parameters in COVID-19 infection

CBC is the most frequent test used in clinical practice, the use is more prominent in the COVID-19 era, especially in the differential count used to evaluate its role in immunity. In the daily practice with automated blood cell counter, although many useful parameters can be analyzed using a single sample of blood not every clinician makes use of its interpretation for patient care. Each parameter or combine parameters can give hints about the patient's condition. Lymphocyte and necrophile counts were used as a factor in distinguishing the COVID-19 infection from other viral and bacterial infection.[22]

During the COVID-19 pandemic, WBC counts plays an important role, especially in lymphocytes and neutrophils. It was observed that 25-33.7% of the patients suffered from leukopenia with a WBC count below 4000/uL. In COVID-19 infection 83.2% of patients also had distinct lymphopenia less than 1.500/uL and because of the other infection, the neutrophils increased, which cause the NLR increase in patients with positive PCR for COVID-19. The NLR became the screening instrument for suspicion of COVID-19. In smaller children, the decrease of WBC and lymphocytes were not so low compared to older children or adults. It was related to higher natural killer cells in children compared to adults. The increase of neutrophils correlated with the severity of the disease.[22,23]

Platelet counts in children with COVID-19 infection were reduced more compared to adults. In severe COVID-19 infections, the platelet counts were less than $150x10^3$/mL. Platelet to Lymphocyte Ratio (PLR) marker can also be used to evaluate the degree of infection and is related to cytokine storm in COVID-19 infection, just like NLR. This PLR is rarely mentioned in children, but PLR greater than 126.7 is a predictor of the worsening condition of the patient.

The average hemoglobin in children with COVID-19 was significantly reduced, and those with severe conditions also had lower hemoglobin levels. It was proposed that the cause of lower hemoglobin was related to the increase of ferritin and less iron that can be used by the RBC to produce hemoglobin. It was also reported that this infection also destroys kidney tissues rich in ACE2 receptors, which will eventually reduce RBC production.[22]

CONCLUSION

Hematology is the most common test performed in a clinical laboratory and is also the most used parameter by clinicians, especially pediatricians. An established reference interval for hematological parameters in babies and children in Indonesia would be very useful in the epidemiological or clinical setting. The hematology reference ranges were categorized as babies, children aged 8 to 12, and more than 12 to 15 years old. Some of the pediatric hematology reference values reported slightly differed compared to those used by WHO or reported in other countries, a specific age and ethnic reference value will be beneficial in managing pediatric patients.

REFERENCES

1. Bates I. Reference ranges and normal values. In Bain BJ, Bates I, Laffan MA, Lewis SM(Eds). Dacie and Lewis Practical hematology. 12th Ed., International China Elsevier, 2107; 8-17.
2. Khusun H, Yip R, Schultink W, Dillon HS. World Health

Organization hemoglobin cut-off points for the detection of anemia are valid for an Indonesian population. J Nutrition, 1999; 129: 1669–74.
3. Yalew A, Terefe B, Alem B, Enawgaw B. Hematological reference intervals determination in adults at Gondar University Hospital, Northwest Ethiopia. BMC Res Notes, 2016; 9: 483.
4. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. VMNIS WHO/NMH/NHD/MNM/11.1. Available from https://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf (accessed Sept 5, 2022).
5. Mansyur M, Khoe LC, Karman MM, Ilyas M. Improving workplace-based intervention in Indonesia to prevent and control anemia. J Prim Care Community Health, 2019; 10: 1-7.
6. Timan IS, Aulia D, Atmakusuma D, Sudoyo A, Windiastuti E, Kosasih A. Some hematological problems in Indonesia. Int J Hematol, 2002; 76 (1): 286–290.
7. Funahara Y. Reports field evaluation for anemia Indonesia. Japan Society for the Promotion of Science. Kobe, 2000;
8. Firmansyah A. Reports field survey for children in South Jakarta. Faculty of Medicine University of Indonesia, Jakarta, 2007;
9. Timan IS, Tatsumi N, Funahara Y, Aulia D, Margono S, Sayogo S, *et al.* Anemia in Indonesian school children. ICMR Annals, 2000; 20: 223–230.
10. El Nageh MM, Heuck CC, Appel W, Vandepitte J, Engbaek K, Gibbs WN. Pre-analytical control. In basic of quality assurance for intermediate and peripheral laboratory. WHO Publication, 1992; 1-7.
11. Cho SM, Lee SG, Kim HS, Kim JH. Establishing pediatric reference intervals for 13 biochemical analytes derived from normal subjects in a pediatric endocrinology clinic in Korea. Clinical Biochemistry, 2014; 47: 268–71.
12. Wiesniewska HW, Nowak JK, Dabrowski M, Szydlowska P, Szczepanik M, *et al.* Reliability of complete blood count in children with acute gastroenteritis. Frontiers in Paed, 2021; 9: 1-6.
13. Nurazizah R, Handika RS, Sahiratmadja E, Ismiarto YD, Prihatni D. Concordance test of various erythrocyte indices for screening of beta thalassemia carrier. Indones J Clinical Pathol Med Laboratory, 2022; 28(2): 137-42.
14. Erhabor O, Muhammad HA, Onwuchekwa C, Egenti NB. Interpretation of full blood count parameters in health and disease. Haematol Int J, 2021; 5(1): 10080.
15. Del Cacho BR, del Castilo YM. Anemia classification and diagnosis. Pediatr Integral, 2021; XXV(5): 214-21.
16. Mataos JF, Borges KBG, Fernandes APS, Faria JR, Carvalho MG. RDW as a differential parameter between microcytic anemias in pure and concomitant forms. J Bras Patol Med Lab, 2015; 51: 22-7.
17. Juffrie M, Helmyati S, Hakimi M. Nutritional anemia in Indonesia children and adolescents: diagnostic reliability for appropriate management. Asia Pac J Clin Nutr, 2020; 29(Suppl 1): S18-31.
18. Djaiman SPH, Nurhidayati N, Kristanto AY. Determinants of anemia in children under five years in Indonesia. 2022. Available from: https://ejournal2litbang.kemkes.go.id/index.php/mpk/article. https://doi.org/10.22435/mpk.v32i 1.5360 (accessed Feb 1, 2023).
19. Nah EH, Kim S, Cho S, Cho HI. Complete blood count reference intervals and patterns changesbacros pediatric, adult and geriatric ages in Korea. Ann Lab Med, 2018; 38: 503-11.
20. Benson VS, Hartl S, Barnes N, Galwey N, Dyke MKV, *et al.* Blood eosinophil counts in the general population and airways disease: A comprehensive review and meta-analysis. Eur Respir J, 2022; 59: 2004590.
21. Li K, Peng YG, Yan RH, Song WQ, Peng XX, Ni X. Age dependent changes in total and differential white blood cell count in children. Chinese Med J, 2020; 133(16): 1900-6.
22. Liu L, She J, Bai Y, Liu W. SARS-CoV-2 infection: Differences in hematological parameters between adults and children. Int J Gen Med, 2021; 14: 3035-47.
23. Kosmeri C,Koumpis E, Tsabouri S, Siomou E, Makis A. Hematological manifestations of SARS-CoV-2 in children. Pediatr Blood Cancer, 2020; 67e: 1-9.