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CONTENTS

The Kinetics of White Blood Cells in Acute Dengue Infection

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ABSTRACT

Introduction: Dengue is a mosquito borne viral febrile illness with a high incidence rate of approximately 50 million cases of infection worldwide every year. Dengue virus can infect many cells, e.g. monocytes, dendritic cells, Kuppfer cells, B cells including bone marrow and lung. Leukocytes plays an important roles in eliminating dengue virus especially monocytes. However, dengue virus sometimes attack the monocytes and uses them for replication causing monocyte to undergo apoptosis in order to prevent spreading by certain mechanisms.
Objectives: To explore the difference in white blood cells count in acute dengue patients from day 2 to day 6.
Methods: This research is conducted in a cross sectional observational study method by recording the WBC count, Lymphocytes count, Neutrophils Count, Relative Monocytes Count, and Absolute Monocytes Count from NS-1 positive dengue infection patient using the hematology analyzer. The data was taken from day 2 to day 6 of the fever. One-way ANOVA test was used and a p value < 0.05 was considered as significant.
Results: In this study, there is a significant difference of leukocyte count, relative and absolute lymphocytes count, relative and absolute neutrophils count, and relative and absolute monocytes count from day 2 to day 6 of dengue infection (p value less than 0.05).
Conclusion: Dengue patient have leucopenia on day 2 until day 6 of dengue fever. Lymphocytosis occurs on day 6 of dengue fever. Neutrophils decrease in early infection. Monocytes count is normal in dengue fever, but decrease in DHF

Keywords: dengue infection, leukocyte, lymphocytes, neutrophils, monocytes

INTISARI

Pendahuluan: Dengue adalah demam yang disebabkan oleh virus yang ditularkan melalui nyamuk dengan insidensi tinggi yaitu lebih kurang 50 juta infeksi di seluruh dunia setiap tahunnya. Virus Dengue menginfeksi banyak sel seperti monosit, sel dendrit, sel Kuppfer dan sel limfosit B. Leukosit, khususnya monosit, berperan penting dalam mengeliminasi virus Dengue. Tetapi, virus Dengue mengganggu komunikasi antar sel monosit dan memanfaatkan sel tersebut untuk repikasi virus sehingga menginduksi apoptosis monosit untuk mencegah penyebaran virus melalui beberapa mekanisme tertentu.
Tujuan: Untuk meneliti perbedaan jumlah sel darah putih pada pasien Dengue akut pada hari ke 2 sampai hari ke 6 panas.
Metode: Penelitian ini merupakan penelitian observasional dengan desain potong lintang dengan melihat hitung lekosit, hitung limfosit, hitung netrofil, hitung monosit relatif dan absolut pada pasien Dengue dengan uji NS-1 positif dengan menggunakan Hematology Analyzer. Data diambil dari hari ke

2 sampai hari ke 6 panas. Uji ANOVA digunakan untuk analisa data dengan tingkat kemaknaan p < 0,05.
Hasil: Dalam penelitian ini, terdapat perbedaan yang bermakna pada hitung luekosit, hitung limfosit absolut dan relatif dan hitung monosit absolut dan relatif pada hari ke 2 sampai ke 6 panas (p<0,05).
Simpulan: Pasien dengue mengalami leukopenis pada hari ke 2-6 panas, limfositosis pada hari ke 6 panas dan netropenia pada awal infeksi. Hitung monosit normal pada demam Dengue tetapi menurun pada DHF.

Kata Kunci : infeksi dengue, leukosit, limfosit, netrofil, monosit

INTRODUCTION

Dengue is a mosquito borne viral febrile illness caused by 4 dengue virus serotypes (DENV-1–4) and is currently the most prevalent arthropod borne viral disease worldwide, with an estimation of 100 million infections each year in tropical and subtropical regions[1]. Some 2500 million people, two fifths of the world's populations are now at risk from dengue. WHO currently estimates 50 million cases of dengue infection worldwide every year. During epidemics of dengue, attack rates among susceptible individuals are 40 to 90%. An estimated of 500,000 cases of DHF require hospitalization each year, of which a very large proportion is children[2]. In Indonesia, where more than 35% of the country's population lives in urban areas, 150,000 cases were reported in 2007 (the highest on record) with over 25,000 cases reported from both Jakarta and West Java even the case fatality rate was approximately 1%[3].

The monocyte/macrophage system is involved in acute and chronic inflammatory reactions, playing a major role in host defense. Their functions include antigen presentation, the generation of cytolytic T lymphocyte responses and various accessory cell functions[4]. The main functions of monocytes, namely phagocytosis, antigen presentation and production of cytokines, are mediated by certain surface molecules, such as Fc receptors for IgG, complement receptor CR1, β2-integrins, CD14, IL-2 receptor and MHC class II HLA-DR molecules[5].

Mostly monocytes succeed in their function of eliminating a pathogen from body but sometimes the pathogen attack them and starts using them for its benefit. A number of pathogens including dengue virus (DV), subvert monocytes and use them for their replication increasing severity of the damage to body[2]. In order to prevent the spreading of the virus, the infected monocytes itself will undergo apoptosis which then will be phagocytized by macrophage[6].

Besides, the infected monocytes will act as an APC which involves two mechanisms which are MHC class I and MHC class II. The stimulation of CD8+ will occur in MHC class I and in turn affects the proliferation of T lymphocytes. The stimulated CD8+ will produce two actions namely cytotoxicity and cytolysis to the monocytes-infected-dengue-virus and this causing the monocytes to lyse. In MHC class II, CD4+ will be stimulated and activate the TH1/TH2. TH1 will produce certain mediators such as IFN-γ, IL-2 and CSF. The IFN-γ production will stimulate macrophage and causing it to produce TNF-α and ICAM. Meanwhile, the CSF produced by TH1 will stimulate neutrophils and in association with ICAM produced by macrophage, it will trigger the enzymes lysozymes which will lyses the endothelial cells of the blood vessels resulting in plasma leakage[7].

The aim of this study is to explore the difference in WBC count, relative and absolute lymphocytes count, relative and absolute neutrophils count, and relative and absolute monocytes count in dengue patients from day 2 to day 6.

MATERIALS AND METHODS

This research is conducted in a cross sectional, observational study method at the Dr. Sardjito Hospital from May until October 2010. The inclusion criteria's of subjects were aged more than 14 years old and suspected to have dengue infection with NS-1 positive (+ve). The exclusion criteria's was subjects who were also suffered from other diseases, other than dengue infection.

The blood sample was collected from patients under treatment at Dr. Sardjito Hospital who are suspected to have dengue infection with NS-1 positive (+ve). The blood vein sample is immediately collected prior to admission to the hospital. The blood will be taken aseptically by vena puncture and kept in the K3 EDTA vaccutainer blood collection tube. The blood sample was stored at 20°C-25°C. Then, the sample will be sent to Clinical Pathology Laboratory for complete blood count using hematology analyzer. WBC count, relative and absolute lymphocytes count, relative and absolute neutrophils count, and relative and absolute monocytes count will be recorded from the hematology analyzer data.

WBC count, relative and absolute lymphocytes count, relative and absolute neutrophils count, and relative and absolute monocytes count will be analyzed descriptively and statistically. To know the significant difference of the data from day 2 until day 6, one-way ANOVA as the statistical test was used as we have to compare multiple day from day 2 to day 6. A p value < 0.05 was considered as significant.

RESULTS AND DISCUSSIONS

This study examined the WBC count in patients who were positive with dengue infections. Among the study subjects, there were 36 patients aged more than 14 years old and suspected for dengue with NS-1 positive but only 32 patients who were eligible for this study and have a complete data on the WBC count, relative and absolute lymphocytes count, relative and absolute neutrophils count, and relative and absolute monocytes count from day 2 to day 6 of dengue infection. The results from hematology analyzer were analyzed for descriptive statistics and mean difference was analyzed using one-way ANOVA. The description about the r data showed in Table 2 and Figure 1 – Figure 4. By using a P-P plot, all the data are normally distributed.

Table 1. Classification of Dengue Infection Among Subject

Type of Dengue	Frequency (n)	Percentage (%)
DF	17	53.1
DHF	15	46.9
Total	32	100

Table 1 shows that 53.1% of the subjects were classified as dengue fever and 46.1% were dengue hemorrhagic fever.

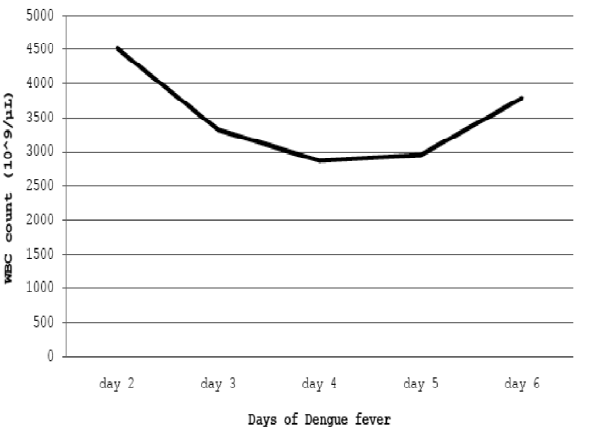

Figure 1. WBC Count of day 2 to day 6 of dengue fever [normal range: 4,000-11,000 × 10^9/µL]

Table 2. Mean of White Blood Cells' Count From Day 2 to day 6

	Day 2	Day 3	Day 4	Day 5	Day 6
	Mean (Standard Deviation)				
WBC	4518.8	3322.6	2867.2	2953.8	3790.9
	(1765.5)	(1217.8)	(1361.5)	(1462.3)	(1671.8)
Lymph.	23.0	28.1	35.3	35.8	46.0
(%)	(10.4)	(11.1)	(11.7)	(10.9)	(10.6)
Neut.	70.5	58.6	55.6	51.3	40.0
(%)	(13.3)	(15.7)	(11.7)	(15.9)	(15.5)
Mono.	6.6	11.4	9.0	11.3	12.6
(%)	(7.4)	(6.2)	(4.9)	(5.7)	(6.6)
Abs.	935.7	1007.9	996.0	1087.6	1740.6
Lymph.	(342.6)	(680.6)	(565.7)	(771.2)	(897.1)
(10^9/µL)					
Abs.	3281.6	1965.3	1611.9	1467.4	1522.0
Neut.	(1632.4)	(812.3)	(971.8)	(727.2)	(955.4)
(10^9/µL)					
Abs.	296.9	402.1	245.5	327.3	453.3
Mono.	(365.7)	(287.6)	(162.1)	(218.4)	(274.6)
(10^9/µL)					

Table 2 shows the description for mean of each parameters from day 2 to day 6. Figure 1 shows the kinetics of WBC count which is normal on day 2, and it is decreasing to below its normal value on day 3 until day 6. The ANOVA analysis of WBC count is significant with the p- value 0.000 (<0.05). Table 3 shows the multiple comparison of the data that are significant with p-value <0.05.

Table 3. Multiple Comparisons (LSD) – WBC Count from Day 2 to Day 6

(I)Days	(J)Days	Significant
2	3	0.002
5	6	0.028

The lymphocytes count was analyzed and shown in Table 2. The relative lymphocytes count is in its normal range on day 2 until day 5, but slightly increases on day 6 as shown in figure 2. However, the absolute lymphocytes count is below its normal value from day 2 to day 5 and only rise to normal on day 6 as shown in Figure 2.

The ANOVA of lymphocytes count is significant with the p-value for relative lymphocytes is 0.000 and absolute lymphocytes is 0.000 (<0.05). Table 4 and Table 5 show the multiple comparison of the data that are significant with p-value <0.05.

Table 4. Multiple Comparisons (LSD)–Relative Lymphocytes Count from Day 2 to Day 6

Days	Days	p value
3	4	0.009
5	6	0.000

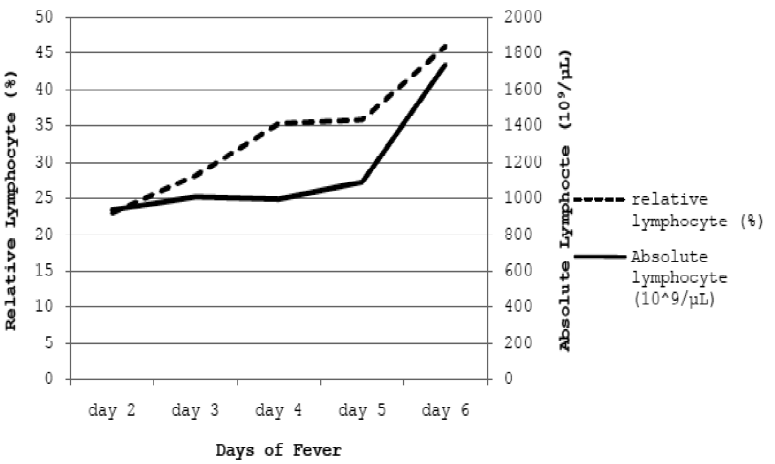

Figure 2. Lymp hocytes Count of day 2 to day 6 of dengue fever [normal range: 1. relative lymphocytes (20-45%), 2. absolute lymphocytes (1500-4500 × 10^9/µL)]

Table 5. Multiple Comparisons (LSD)-Absolute Lympho-cytes Count from Day 2 to Day 6

Days	Days	p value
5	6	0.000

Table 6. Multiple Comparisons (LSD) – Relative Neutrophils Count from Day 2 to Day 6

Days	Days	p value
2	3	0.001
5	6	0.002

Table 7. Multiple Comparisons (LSD) – Absolute Neutrophils Count from Day 2 to Day 6

Days	Days	p value
2	3	0.000

Table 8. Multiple Comparisons (LSD) - Relative Monocytes Count from Day 2 to Day 6

Days	Days	Significant
2	3	0.002

Table 9. Multiple Comparisons (LSD) - Absolute Monocytes Count from Day 2 to Day 6

Days	Days	Significant
3	4	0.022

Based the data from the Figure 3, the relative neutrophils count is decreasing from day 2 until day 6 but if based on the normal range, the relative neutrophils count is in normal value for day 2 until day 6. This is the same with the absolute neutrophils count where the kinetics is decreasing but the value for absolute neutrophils is normal on day 2 but neutropenia occurs on day 3 until day 6.

The ANOVA of neutrophils count is significant with the p-value for relative neutrophils is 0.000 and

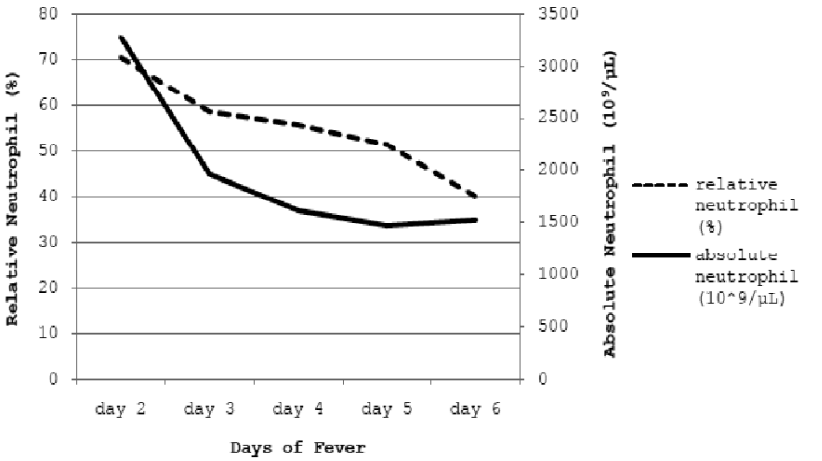

Figure 3. Neutrophils Count of day 2 to day 6 of dengue fever [normal range: 1.relative neutrophils count (40-75%), 2. absolute neutrophils count (2000-7500 × 10^9/µL)]

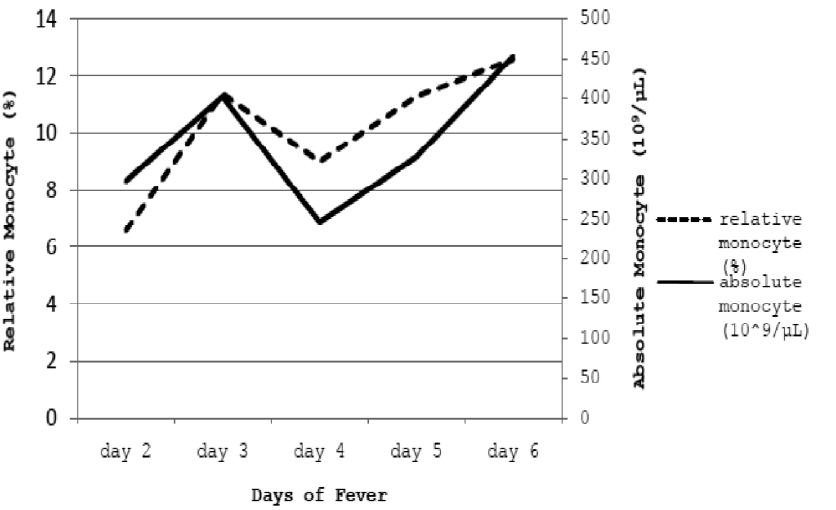

Figure 4. Monocytes Count of day 2 to day 6 of dengue fever [normal range: 1.relative monocytes (2-10%), 2.absolute mono-cytes (normal range: 200-800 × 109/µL)]

absolute neutrophils is 0.000(<0.05). Table 6 and Table 7 show the multiple comparison of the data that are significant with p-value <0.05.

The description of relative monocytes count in this study was shown in Table 2. From Figure 4, the kinetics of monocytes count is in the normal value for day 2, slightly increase on day 3, return back to its normal value on day 4, and slightly increase on day 5 until 6.

The kinetics of absolute monocytes count is almost similar with the relative count which is increases from day 2 to day 3, decreases on day 4

and increase again until day 6 as shown in Figure 5. However, the absolute monocytes count in the data is in the normal range from day 2 until day 6.

The ANOVA of monocytes count is significant with the p-value for relative monocytes is 0.001 and absolute monocytes is 0.020 (<0.05). Table 8 and Table 9 show the multiple comparison of the data that are significant with p-value <0.05.

All the data from this research were taken from day 2 and not from day 1 since intracellular and extracellular infectious viruses were synthesized and released after 12 h of infection. This event is followed by a log phase of growth, and peak viral yields were detectable at about 36 to 48 hours post infection[21]. By taking the data from day 2, we can avoid getting a result that is insignificant.

Our study showed that there is a significant difference of WBC count between days. WBC count was normal on day 2 (4518) and leukopenia was detected on day 3 (3322) until day 6 (3790) with an increase from day 5-6 to its normal value. The result is similar with the study done by Kularatne et al. where the white blood cell count (WBC) in the study showed a significant leukopenia starting from the second day of illness with a nadir on the fifth day[5]. The distribution of mean total white blood cell counts (WBC) showed a trend towards leucopenia with lowest count on the 5th and 6th days of fever. In addition, the study done by Lei et al. (2008) stated that WBC count is also increase on day 5-6 as analysis of the clinical blood cell count of the study showed that increase of immature neutrophils developed at fever day 5-6[11].

In comparison, the relative neutrophils count from our data is in its normal value from day 2-6, but the kinetics of it is decreasing significantly and gradually from 70.5% on day 2 to 40.0% on day 6 (normal range: 40-75%). However, the kinetics of absolute neutrophil is also decreasing but it is in its normal value only on day 2, and neutropenia for day 3 until 6. The decreasing of neutrophils affect the WBC count as 40-75 % of WBC consists of neutrophils[8]. A study done by Mathew et al. (2010) also showed that there is a decrease of absolute neutrophils count in acute dengue infection[14]. The study also suggested that leukopenia is thought to be related to bone marrow suppression induced by Dengue Virus resulting in neutropenia. A review of experimental dengue infections of volunteers and histopathological studies of bone marrow from patients with severe dengue virus infection done by La Russa and Innis (2005) suggests that marrow suppression evolves rapidly through several phases i.e. (1) onset of marrow suppression within 3-4 days of infection; (2) onset of host inflammatory responses in the marrow and of fever shortly thereafter; (3) occurrence of a neutrophils nadir on the fourth to fifth day after onset of fever; (4) almost simultaneously, immune activation sufficient to neutralize viraemia and accelerate elimination of infected cells; (5) remission of symptoms; and (6) resolution of cytopenias. Moreover, in vitro study showed an early blast cells as well as the more differentiated hematopoietic elements were abortively infected, killed and eliminated by phagocytosis by specialized marrow macrophages called dendritic cells. The ARC from stroma rather than haematopoietic precursors was productively infected. When ARC was infected, stroma failed to support hematopoiesis which in turn causing leucopenia[15].

As for lymphocyte count, our data shows the kinetics of relative lymphocytes count increase from day 2 (22.9%) to day 6 (46.0%) but the value from day 2-5 still in its normal range with a slight increase on day 6. A research done by Malavige et al. (2004) supported our data as they showed that the onset of dengue is leucopenia[16]. The initial leucopenia is followed by a relative lymphocytosis (with more than 15% atypical lymphocytes) towards the end of the febrile phase. Gubler (1998) also gave the same result of clinical laboratory findings associated with dengue

fever include a neutropenia followed by a lymphocytosis, often marked by atypical lymphocytes[17].

At the earliest stages of dengue infections, preformed dengue virus-specific antibodies increase the number of virus-infected monocytes. As a result, the number of cells presenting dengue viral antigens to T lymphocytes is increased. In the middle stages of infection, the level of T-lymphocyte activation is markedly increased, reflecting increased antigen presentation, increased frequency of dengue virus-specific T lymphocytes in secondary infection, and more rapid activation and proliferation of memory T lymphocytes[13]. Apart from that, antibody dependent enhancement (ADE) is thought to play a key part in the pathogenesis of severe dengue infections. During secondary dengue infections, antibodies already present in the patient form complexes with the dengue virus. The Fc portion of these antibodies can then bind to FccRI and FccRII bearing cells and result in an increased number of cells being infected by the dengue virus. Antibody dependent enhancement is found to occur only in the presence of subneutralising concentrations of dengue antibodies. DEN-1 immune sera at 1:250 dilution (subneutralising titre), but not at 1:10 dilution, enhances DEN-2 infection of mononuclear leucocytes, in turn resulting in increased lymphocyte proliferation and decreased interferon-c (IFN-c) production[11].

However, the absolute lymphocyte have a contradict result compared that of the previous study because in this study, the lymphocytes is decrease (lymphocytopenia) on day 2 until day 5, and go back to normal on day 6. Based on the immunopathogenesis of dengue, this condition is not supposed to occurs. The possible reason for the lymphocytopenia is maybe most of the patient are taking medication containing steroid. As the study done by Elmadjian and Pincus (1945) stated that there is a reduction in the absolute count of lymphocyte in patient taking medication such as adrenal cortical extract, adrenal steroid and adrenotropic hormone[19]. On the other hand, Kronfol et al. (1985) showed that depression can be the reason of lymphocytopenia as depression produce cortisol which is a steroid hormone; a hormone that able to suppress the immune system[20]. The study also showed there is a significant differences in the percentages of both neutrophils and lymphocytes as well as the absolute number of lymphocytes in depress patient compare to non-depress patient. These differences were mostly due to significantly lower lymphocyte percentages and absolute counts of the lymphocyte.

The difference for both relative and absolute monocytes count from day 2 to day 6 is different significantly. The relative monocytes count is normal on day 2 (6.56%), with slightly increase on day 3 (11.36%), go back to normal value on day 4 (8.99%) and increase a bit until day 6 (12.59%). As for the absolute monocytes count, the kinetics pattern is the same as relative monocytes count, but the data from day 2-6 is still in its normal value. Mathew et al. (1999) stated study days 2 or 3 represent days of dengue hemorrhagic fever when the absolute monocytes counts drop to the lowest levels[14]. Patients are leukopenia for several days during acute dengue virus infection with a decrease in the absolute number of neutrophils and monocytes. This decrease is temporary, and monocytes counts return to normal within a few days. So, the reason the absolute monocytes count in our data is still normal may be most of our subjects are suffer from dengue fever and not dengue hemorrhagic fever. However, in the study done by Chen and Wang (2002) stated that after 2 days of cultivation, these cells began to enlarge and make contact, with frequent formation of cell clumps. Around 5 to 6 days of culture, the cell monolayers reached subconfluence, and sub-populations of cells began to fuse and form small multinucleated giant cells (MNGC), which contained about 2 to 3 nuclei per cell[21]. After 6 to 7 days, the number of MNGC increased markedly and the cell monolayer reached confluence. Afterwards, the size

of MNGC enlarged progressively, and the number of nuclei in a single MNGC increased from 2 to more than 10, although some monocytes remained mononuclear. At about 10 days after culture, huge MNGC which possessed about 40 to 50 nuclei formed and were osteoclast-like in morphology. At this point, the monocytes begin to lyse.

As for the kinetics of the monocyte, it increase from day 2 to 3, this is because when the monocyte is infected by some pathogen, it will secrete large amounts of protein, namely TNF, interleukin-1, interferon, protein coagulation, cell adhesion proteins, protease, and cytokines (G-CSF and GM-CSF). The production of G-CSF and GM-CSF will stimulate the common myeloid precursor which will then develop to become monocytes. The kinetics of monocytes decrease from day 3 to 4, Kanakoudi-Tsakalidou et al. (2000) stated that the most probable explanations for this finding could be a redistribution of monocytes with migration of the activated monocytes to the injured tissues and become macrophage[10]. So, this will decrease the total number of monocytes in the circulation.

From the immunopathogenesis of dengue infection, the serotype cross-reactive antibodies from the previous dengue infection will bind to virions without neutralization and enhance the entry of virus into monocytes. The number of virus-infected monocytes increases. As a result, the level of dengue virus- specific T cell activation is markedly enhanced. The T cells, especially the cross-reactive T cells, produce cytokines such as IFN-g, IL-2 and TNF-alpha and lyse dengue virus-infected monocytes. TNF-alpha is also produced by activated monocytes. Dengue virus can infect immature dendritic cells, monocytes, as well as B cells. Monocytes were the major target for dengue virus, but it will undergo apoptosis to prevent the spread of virions. It also decrease the viral-induced inflammation because the apoptotic cells will be phagocytosized by macrophages. This in turn causes the monocytes' count to decrease[6].

CONCLUSIONS

Based on the explanation above, we can conclude that there is a difference in the kinetics of white blood cells from day 2 to day 6 of acute dengue infection. WBC is normal on day 2, but decreases to below the normal value on day 3-6. Relative lymphocyte is normal on day 2-5, with lymphocytosis occurs on day 6. Neutrophils is normal on day 2, with neutropenia occurs on day 3-6. Monocytes count is normal from day 2-6.

SUGGESTIONS

We can have more accurate result if the data collection of WBC count, lymphocyte count, neutrophils count and monocytes count in all blood samples were taken in a period until the participants are recovered completely. It is better if we can include the race classification in the research since previous study showed there is different in the epidemiology between the races.

REFERENCES

1. Andreoli TE, Carpenter CCJ, Griggs RC, Loscalzo J. CECIL Essentials of Medicine. 6th Ed. Saunders, Philadelphia, 2004.
2. Baratawidjaja KG. Imunologi Dasar. Edisi 7. Balai Penerbit Fakultas Kedokteran UI, Jakarta, 2006.
3. Dorland's Illustrated Medical Dictionary. 31st Edition, Saunders, 2007;1100.
4. Kalayanarooj S, Vaughn DW, Nimmannitya S, Green S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachu-eke S, Kiatpoljoj S, Innis BL, Rothman AL, Nisalak A, and Ennis FA. Early Clinical and Laboratory Indicators of Acute Dengue Illness. J Infect Dis, 1997; 176(2): 313-21.
5. Kularatne SAM, Gawarammana IB, and Kumarasiri PRV. Epidemiology, Clinical Features, Laboratory Investigations and Early Diagnosis of Dengue Fever In Adults : A

Descriptive Study In Sri Lanka. South East Asian J Trop Med, 2005;36(3):686-92.

6. Kyle JL, Beatty PR, and Harris E. Dengue Virus Infects Macrophages and Dendritic Cells in a Mouse Model of Infection. J Infect Dis, 2007; 195(12):1808-17.
7. Chaturvedi UC, Nagar R, & Shrivastava R. Macrophage & dengue virus: Friend or foe? Indian J Med Res, 2006;124(1):23-40.
8. Anonim. Dengue Guidelines for Diagnosis, Treatment, Prevention & Control. Chapter 1: Epidemiology, burden of disease and transmission. WHO, 2009;5.
9. Stevenson HC, Dekaban GA, Miller PJ, Benyajati C, and Pearson ML. Analysis of human blood monocyte activation at the level of gene expression. Expression of alpha interferon genes during activation of human monocytes by poly IC/LC and muramyl dipeptide. J Exp Med, 1985;161(3):503-13.
10. Kanakoudi-Tsakalidou F, Debonera F, Agakidou VD, Sarafidis K, Tzimouli V, Taparkou A, & Kremenopoulus G. Flow cytometric measurement of HLA-DR expression on circulating monocytes in healthy and sick neonates using monocyte negative selection. Clin Exp Immunol, 2000;123(3):402-7.
11. Lei HY, Huang KJ, Lin YS, Yeh TM, Liu HS, and Liu CC. Immunopathogenesis of Dengue Hemorrhagic Fever. Am J Infect Dis, 2008; 2-4.
12. Kurane I, Dai LC, Livingston PG, Reed E, and Ennis FA. Definition of an HLA-DPW2-Restricted Epitope on NS3, Recognized by a Dengue Virus Serotype-Cross-Reactive Human CD4+ CD8- Cytotoxic T- Cell Alone. J Virol, 1993;67(10):6285-8.
13. Longmore M, Wilkinson I, Turmezei T, Cheung CK. The Differential White Cell Count. In: Oxford Handbook of Clinical Medicine Seventh Edition. Oxford University Press, 316-7.
14. Mathew A, Kurane I, Green S, Vaughn DW, Kalayanarooj S, Suntayakorn S, Ennis FA, and Rothman AL. Impaired T Cell Proliferation in Acute Dengue Infection. J Immunol, 1999; 162(9):5609-15.
15. La Russa VF, and Innis BL. Mechanisms of Dengue Virus-Induced Bone Marrow Suppression. Baillieres Clin Haematol, 1995; 8(1):249-70.
16. Malavige GN, Fernando S, Fernando DJ, Seneviratne SL. Dengue Viral Infections. Post-grad Med J, 2004;80(948):588-601.
17. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. Clin Microbiol Rev, 1998;11(3):480-96.
18. da Fonseca BA, and Fonseca SN. Dengue Virus Infections. Curr Opin Pediatr, 2002;14(1):67-71.
19. Elmadjian F, and Pincus G. The Adrenal Cortex and the lymphocytopenia of stress. Endocrinology, 1946;39(5):293-9.
20. Kronfol Z, Nasrallah HA, Chapman S, House JD. Depression, Cortisol Metabolism and Lymphocytopenia. J Affect Disord, 1985; 9(2): 169-73.
21. Chen YC, and Wang SY. Activation of Terminally Differentiated Human Monocytes/Macrophages by Dengue Virus: Productive Infection, Hierarchical Production of Innate Cytokines and Chemokines, and the Synergistic Effect of Lipopolysaccharide. J Virol, 2002;76(19):9877-87.
22. Male D, Brostoff J, Roth DB, Roitt I. Immunology 8th Ed. Mosby Elsevier, 2006;23.

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Sample References

Scientific Journal

1. *Standard journal article*

   You CH, Lee KY, Chey RY, Menguy R. Electro-gastro-graphic study of patients with unexplained nausea, bloating and vomiting. Gastroenterology 1980; 79(2):311-14.

   Goate AM, Haynes AR, Owen MJ, Farral M, James LA, Lai LY, et al. Predisposing locus for Alzheimer's disease on chromosome 21. Lancet 1989;1:352-55.

2. *Organization as author*

   The Royal Marsden Hospital Bone-marrow Transplantation. Team. Failure of syngeneic bone-marrow graft without preconditioning in post-hepatitis marrow aplasia. Lancet 1977;2:742-44.

3. *No author given*

   Coffee drinking and cancer of the pancreas [editorial]. BMJ 1981;283-628.

4. *Article not in English*

   Massone L, Borghi S, Pestarino A, Piccini R, Gambini C. Localisations palmaires purpuriques de la dermatite herpetiforme. Ann Dermatol Venereol 1987;114:1545-47.

5. *Volume with supplement*

   Magni F, Rossoni G, Berti F, BN-52021 protects guinea-pig from heart anaphylaxis. Pharmacol Res Commun 1988;20 Suppl 5:75-78.

6. *Issue with supplement*

   Gardos G, Cole JO, Haskell D, Marby D, Paine SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

7. *Volume with part*

   Hanly C. Metaphysics and innateness: a psychoanalytic perspective.Int J Psychoanal 1988;69(Pt 3):389-99.

8. *Issue with part*

   Edwards L, Meyskens F, Levine N. Effect of oral isotretinoin on dysplastic nevi. J Am Acad Dermatol 1989;20(2 Pt 1):257-60.

9. *Issue with no volume*
   Baumeister AA. Origins and control of stereotyped movements. Monogr Am Assoc Ment Defic 1978; (3):353-84.
10. *No issue or volume*
    Danoek K. Skiing in and through the history of medicine. Nord Midicinhist Arsb 1982;86-100.
11. *Pagination in roman numerals*
    Ronne Y. Ansvarfall. Bloodtransfusion till fel patients. Vard-facket 1989;13:XXVI-XXVII.
12. *Type of article indicated as needed*
    Spargo PM, Manners JM, DDAVP and open heart surgery [letter]. Anaesthesia 1989;44: 363-64.
    Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondii [abstract]. Clin Res 1987; 35:475A.
13. *Article containing retraction*
    Shishido A. Retraction notice: Effect of platinum compounds on murine lymphocyte mitogenesis [Retraction of Alsabti EA, Ghalib ON, Salem MH. In: Jpn J Med Sci Biol 1979; 32:53-65). Jpn J Med Sci Biol 1980;33:235-37.
14. *Article retracted*
    Alsabti EA, Ghalib ON, Salem Mh. Effect of platinum compounds on murine lymphocyte mitogenesis [Retracted by Shishido A. In: Jpn J Med Sci Biol 1980;33:235-7]. Jpn J Med Sci Biol 1979;32:53-65.
15. *Article containing comment*
    Piccoli A, Bossatti A. Early steroid therapy in IgA neuropathy: still open question [comment]. Nephron 1989;51:289-91.
16. *Article in comment*
    Kobayashi Y, Fujii K, Hiki Y, Tateno S, Kurokawa A, Kamiyama M. Steroid therapy in IgA nephropathy: a retrospective study in heavy proteinuric cases [see comments]. Nephron 1988;48:12-7. Comment in: Nephron 1989;51:289-91.
17. *Article with published erratum*
    Schofield A. The CAGE questionnaire and psychological health [published erratum appears in Br J Addict 1989;84:701]. Br J Addict 1988;83:761-64.

Books and Other Monographs

18. *Personal author(s)*
    Colson JH, Armour WJ. Sports injuries and their treatment. 2nd rev. ed. London: S. Paul, 1986.
19. *Editor(s) as author*
    Diener HC, Wilkinson M, editors. Drug-induced headache. New York: Springer-Verlag, 1988.
20. *Organization(s) as author*
    Virginia Law Foundation. The medical and legal implications of AIDS. Charlottesville: The Foundation, 1987.
21. *Chapter in a book*
    Winstein L, Swartz MN. Pathologic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, editors. Pathologic Physiology, mechanisms of disease. Philadelphia: Saunders, 1974:457-72.
22. *Conference proceedings*
    Vivian VL, editor. Child abuse and neglect: a medical community response. Proceedings of the First AMA National Conference or Child Abuse and Neglect; 1984 Ma 30-31; Chicago. Chicago: American Medical Association, 1985.
23. *Conference paper*
    Harley NH. Comparing radon daughter dosimetric and risk models. In:Gammage RB, Kaye SV, editors. Indoor air and human health. Proceedings of the Seventh Life Sciences Symposium; 1984 Oct 29-31; Knoxville (TN). Chelsea (MI):Lewis, 1985:69-78
24. *Scientific or technical report*
    Akutsu T. Total heart replacement device. Bethesda (MD): National Institutes of Health. National Heart and Lung Institute; 1974 Apr. Report No.:NIH-NIHI-69-2185-4.
    Disertasi Youssef NM. School adjustment of children with congenital heart disease [dissertation]. Pittsburg (PA): Univ. of Pittsburg, 1988.

25. *Dissertation*
    Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [Dissertation]. St Lucia, Qld: University of Queensland; 2007.
26. *Patent*
    Harred JF, Knight AR, McIntyre JS, inventors. Dow Chemical Company, assignee. Epoxidation process. US patent 3,654,317, 1972 Apr 4.

Other Published Material

27. *Newspaper article*
    Resberger B, Specter B. CFCs may be destroyed by natural process. The Washington Post 1989 Aug 7;Sect. A:2(col. 5).
28. *Audiovisual material*
    AIDS epidemic: the physician's role [videorecording]. Cleveland (OH): Academy of Medicine of Cleveland, 1987.
29. *Computer program*
    Renal system [computer program]. MS-DOS version. Edwardsville (KS): Medi-Sim, 1988.
30. *Legal material*
    Toxic Substances Control Act: Hearing on S. 776 Before the Subcomm. on the Environment of the Senate Comm. on Commerce, 94th Cong., 1st Sess. 343(1975).
31. *Map*
    Scotland [topographic map]. Washington: National Geographic Society (US), 1981.
32. Dictionary or Encyclopaedia
    Ectasia. Dorland's illustrated medical dictionary. 27th ed. Philadelphia: Saunders, 1988: 527.
33. *Classic material*
    The Winter's Tale: act 5, scene I, lines 13-16. The complete works of William Shakespeare. London: Rex, 1973.
34. *In press*
    Lillywhite HB, Donald JA. Pulmonary blood flow regulation in an aquatic snake. Science. In press.

Electronic Material

35. *Journal articel in the internet*
    Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis [serial online] 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm
36. *Monograph in electronic format*
    CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0 San Diego: CMEA; 1995.
37. Computer program
    Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational System; 1993.

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