# Kinetics of CD69 Expression on Natural Killer Cells During Acute Phase of Dengue Infection

**Marshita Binti Kamarudin[1], Umi Solekhah Intansari[2*], Ida Safitri Laksanawati[3]**
[1]Undergraduate Program of Medicine, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia; [2]Department of Clinical Pathology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia; [3]Department of Pediatric, DR. Sardjito General Hospital, Yogyakarta, Indonesia.

Corresponding author: umintasari2003@yahoo.com

## ABSTRACT

**Introduction:** Dengue infection is major annual public health problem in Indonesia. NK cells have a role in cellular immunity to viral infection, however only a few studies of NK cells and were conducted in vivo especially in Indonesia.
**Objectives:** To explore the kinetics of CD 69 expression on NK cells during the acute phase of dengue infection.
**Methods:** Observational cohort study in Dr. Sardjito Hospital was conducted. Clinical data and laboratory data was collected to measure the number of activated NK cells (CD69) using flowcytometry. The percentage of CD69 then calculated using non- parametric test (Kuskal-Wallis Test) and Student t-test. The fluorescence intensity of CD69 was also analyzed.
**Results:** The mean of activated NK cells (CD69) percentage was higher during the early days of acute phase (day 2 to day3), and continuously declined until the seventh day but statistically they were not significant. Fluorescence intensity of CD69 showed its peak during the fifth day of fever.
**Conclusion:** CD69 expression on activated NK cells were increased during the early days (day 2-day 3) of acute fever but decreased after that (day 4-day 7). The highest intensity of CD69 expression was on the fifth day of fever.

**Keywords:** Dengue infection, kinetics, NK cell, CD69, cellular immune response, acute phase, adults.

## INTISARI

**Pendahuluan:** Infeksi Dengue adalah masalah kesehatan masyarakat utama tahunan di Indonesia. Sel-sel NK memiliki peran dalam imunitas seluler terhadap infeksi virus, namun hanya beberapa studi sel NK dan dilakukan in vivo khususnya di Indonesia.
**Tujuan:** Untuk mengetahui kinetika ekspresi CD 69 pada sel NK selama fase akut infeksi dengue.
**Metode:** studi kohort observasional di Rumah Sakit Dr Sardjito dilakukan. Data Klinis dan laboratorium dikumpulkan dan selanjutnya jumlah sel NK teraktivasi dihitung (CD69) menggunakan flowcytometry. Persentase CD69 kemudian dihitung menggunakan uji non-parametrik (Kuskal-Wallis Test) dan Student t-test. Intensitas fluoresensi CD69 juga dianalisis.

**Hasil:** persentase Rerata sel NK teraktivasi (CD69) lebih tinggi pada hari-hari awal fase akut (hari 2 sampai hari ke 3), dan terus menurun setelah itu sampai hari ketujuh tetapi secara statistik tidak signifikan. Intensitas fluoresensi dari CD69 menunjukkan puncaknya pada hari kelima demam.
**Simpulan:** Ekspresi CD69 pada sel NK aktif meningkat selama hari-hari awal (hari 2 -3) demam akut tetapi menurun setelah itu (hari 4 hari 7). Intensitas tertinggi ekspresi CD69 pada hari kelima demam.

**Kata kunci:** infeksi Dengue, kinetika, sel NK, CD69, respon imun seluler, fase akut, dewasa.

## INTRODUCTION

Dengue has been called the most important mosquito-transmitted viral disease in terms of morbidity and mortality. Dengue fever is a benign acute febrile syndrome occurring in tropical regions. In a small proportion of cases, the virus causes increased vascular permeability that leads to a bleeding diathesis or disseminated intravascular coagulation (DIC) known as dengue hemorrhagic fever (DHF). Dengue infection (DI) is amongst the most important emerging viral diseases transmitted by mosquitoes to humans, in terms of both illness and death[2]. Secondary infection by a different dengue virus serotype has been confirmed as an important risk factor for the development of DHF[1].

Dengue infection continues causing the most frequent infectious disease in Indonesia[3,4]. DHF epidemics were reported in the Indonesian cities of Surabaya and Jakarta but since then, outbreaks of the disease have spread to involve most of the major urban areas in Indonesia, as well as some of the rural areas of the country[4,5,6].

Dengue virus infection recently is the most common cause of arboviral infection disease in the world, with an estimated annual occurrence of 100 million cases of dengue fever and 250.000 cases of DHF (dengue hemorrhagic fever) and a mortality rate of 25.000 per year[7]. In Indonesia, dengue-2 virus (DENV2) was identified most frequently among the symptomatic cases and it is assumed that this was the predominant serotype in Indonesia[4]. But now, dengue virus serotype 3 (DEN-3) has been recognized as the predominant serotype in many recent epidemic occurrences of DHF in Indonesia[8].

Dengue infection is a major annual public health problem causing cyclical epidemics in urban centres in Indonesia. The disease is a leading cause of hospitalization and death among children. Epidemics have been consistently documented to occur between January and June. Attack rates among susceptibles are often 40 - 50%, but may reach 80 - 90%. The maximum cases recorded during epidemics in previous years were over 40,000 in 1988, 1996, 1998, 2001, 2003 and 2004, reaching 72,133 in 1998 and 69,017 in 2004[9].

There were 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. The case-fatality rate was approximately 1%[10]. In Indonesia, surveillance data from 1975 to 1984 showed an increase in incidence rates among young adults in Jakarta as well as in the provincial areas[4].

The pathogenesis of DHF is poorly understood. The favored antibody dependent enhancement theory of DHF pathogenesis is

based on epidemiologic studies demonstrating that prior infection with a different viral serotype predisposes to DHF[11] whereas circulating dengue virus spesific IgG antibodies represent major risk factor for DHF and cytotoxic T cells may trigger DHF[12]. It is also likely that dengue virus specific cytotoxic T cells are important for recovery from dengue virus infection. However, only a few of study know about this mechanism in vivo[13].

The manifestation of dengue infections vary from asymptomatic until dengue live threatening condition, dengue shock syndrome. It is hypothesized that immune responses including T-cell-mediated immunopathogenesis contribute to severity of the disease[14].

In the non specific immune response, natural killer (NK) cell play an important role. NK cells are an early component of the host response to viral infection in dengue infection as the act as effector cells lysing dengue virus infected cells. Primary dengue infection induces serotype-specific cross reactive CD4 and CD8 memory cytotoxic t-lymphocytes and also CD69 marker on NK cells[15].

There are several previous studies have been conducted to study the role of NK cells in vivo during dengue infection[15,16,17]. However, more data is needed to increase the under-standing of dengue infection pathogene-sis. Our present study goal is to know the activity of NK cells in dengue infection patients regarding CD 69 marker on NK cells on daily kinetics. This study aimed to explore the kinetics of CD 69 expressi-on on NK cells during the acute phase of dengue infection.

## MATERIALS AND METHODS

This research was an observational cohort study on dengue infection patients. Sample was obtained from admitted patient in RSUP Dr. Sardjito, Yogyakarta and taken in period from March until June of 2009. This study was conducted at Department of Internal Medicine and Department of Pediatric, RSUP Dr. Sardjito, Yogyakarta for taking the clinical data and blood sample and Clinical Pathology Laboratory to analyze samples.

Patients with diagnosis of dengue fever or dengue hemorrhagic fever hospitalized in the Dr. Sardjito Hospital were enrolled in this study. Dengue infection was confirmed using NS1 test. The severity of disease was graded according to WHO criteria for dengue hemorrhagic fever. The confirmed diagnosis adjusted by serology examination.

Inclusion criterias of the subjects were dengue fever or dengue hemorrhagic fever patients admitted to RSUP Dr. Sardjito who has given their informed consent to participate in this study. For pediatric patients, informed consent was obtained from their parents or guardian. The participants are e”14 years old, tested positive with NS1 test and did not have any other infectious disease.

NK Cell examination was performed using flow cytometry; Activation of NK cell was detected by measuring CD69 expression on NK cell membrane using CellQuest program.

Data were collected through clinical data while monitoring the patients and laboratory analysis reports that yield clinical and laboratory findings respectively. The clinical data included name of the patient, age, day of admission and discharge due to fever, and also final grading of disease. Along with detecting relative number of activated NK cell (CD69), the intensity of CD69 expression was also conducted through assessing patient’s blood sample at Clinical Pathology Laboratory.

The Kruskal-Wallis Test was used to analyze the significance in percentage of CD69 expression on activated NK cells and mean or median of CD69 expression. Student t-test was used to analyze the significance in percentage of CD 69 expression on activated NK cells on day by day comparison. A P value less than 0.05 was noted as statistically significant.

## RESULTS AND DISCUSSIONS

NK cells play an important role during the early phase of infection as a non adaptive immune response[18]. The NK cells then will be activated and display CD69 on its surface, this can be measured to see how the percentage change over the acute phase of dengue fever which are measured from second day till seventh day of fever. To study the kinetics of NK cell activation (expression of CD69) during the acute phase of dengue, two analysis were made which are on percentage and median of relative number of CD69.

The number of patients included in this research were 35 patients. The data was taken from dengue fever patients that were at least 14 years old and was taken from March until June of 2009. These data included all types of dengue fever.

From the analysis, the number of patients enrolled in this study was 20 male patients and 15 female patients that gives the percentage distribution of 57.1% and 42.9% respectively as showen in Figure 1.

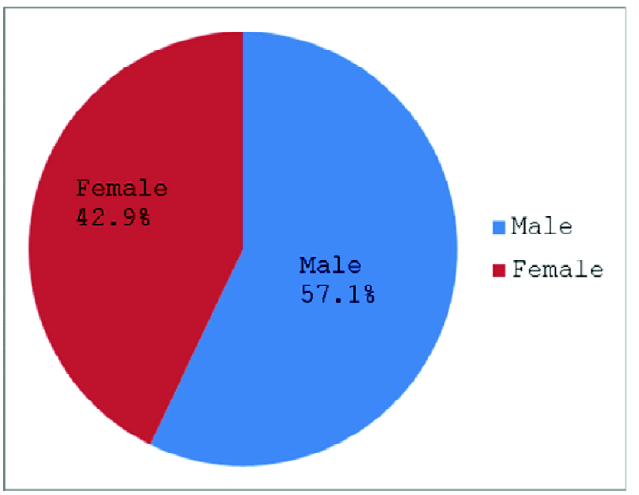


Figure 1. Gender distribution of enrolled patients.

Distribution of age of patients were divided into below 19 years old that comprised of 14 patients (40%), between 19 to 30 years old there were 19 patients (54%), and 2 patients(6%) were above 30 years old as depicted in Figure 2.

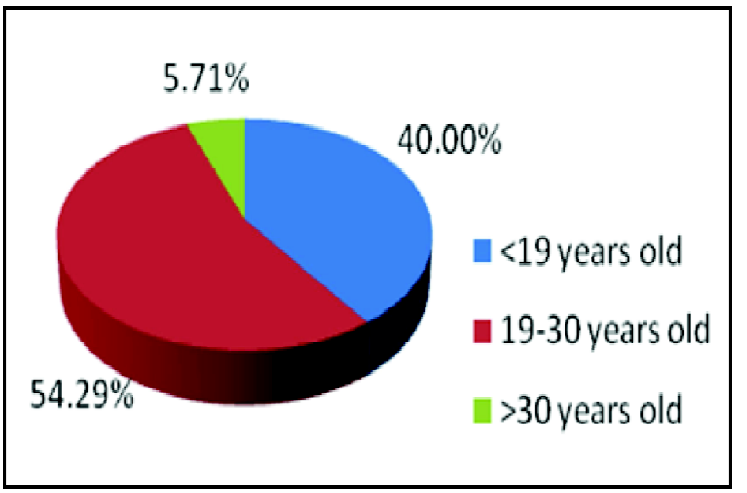


Figure 2. Age distribution of enrolled patients.

There were 20 (57.1%) patients diagnosed as mild dengue infection cases and 15(42.9%) other patients were diagnosed as severe dengue infection cases (Figure 3)

The data provided the number of CD69 from the first day of fever until the eighth day of fever. However, the first day and the eighth day were not included in analysis as the size of the data for both day are smaller compared to the other day which may make the outcome to be biased. However there are still some missing data on second day, sixth day and seventh day as shown on the table 1.

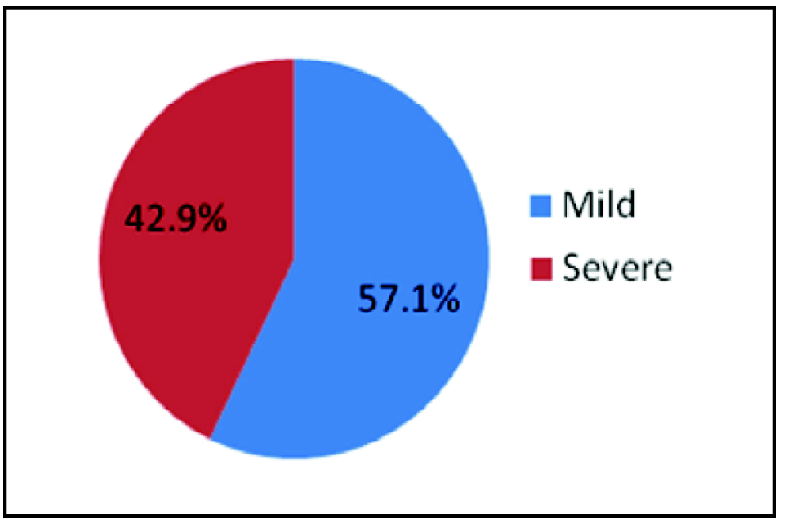


Figure 3. Severity distribution of dengue infection

Table 1. Number of data size based on days.

| Day | N |
|---|---|
| 2 | 31 |
| 3 | 35 |
| 4 | 35 |
| 5 | 35 |
| 6 | 34 |
| 7 | 34 |
| Total | 204 |

**Kinetics of CD69 percentage expression on activated NK cells from day 2 to day 7.**

Percentage of CD69 was observed on daily basis, from day 2 until day 7 of fever. Subsequently, the data was analyzed to find the mean of the CD69 percentage.

Table 2 showed sample size, mean value, minimum value and maximum value of CD69 percentage. Data showed that from the mean value of CD69 percentage, the percentage seem slightly increased during the early days of fever during acute phase which were from day 2 (mean=42.18±15.69) to day 3 (mean= 43.78± 18.73). But it then declined gradually starting from the 4$^{th}$ day of fever until the 7th day of fever. From mean of CD 69 percentage, it showed that the data is coherent with this research hypothesis.

Table 2. Mean value, minimum and maximum value of CD69 percentage

| Day | N | Mean + SD | Minimum | Maximum |
|---|---|---|---|---|
| Day 2 | 31 | 42.18±15.69 | 8.08 | 69.06 |
| Day 3 | 35 | 43.78±18.73 | 12.67 | 73.00 |
| Day 4 | 35 | 41.08±18.95 | 3.14 | 80.00 |
| Day 5 | 35 | 39.83±17.66 | 2.43 | 72.92 |
| Day 6 | 34 | 35.81±17.11 | 6.53 | 62.31 |
| Day 7 | 34 | 34.87±15.42 | 10.00 | 65.73 |
| Total | 204 | 39.58±17.44 | 2.43 | 80.00 |

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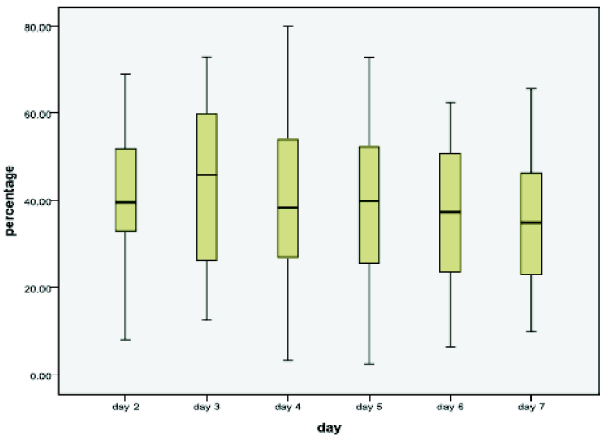


Figure 4. Distribution of CD69 percentage based on the days of fever.

From the box plot graph in Figure 4, it is shown that there is a slight increase of CD69 percentage from day 2 to day 3. This is somewhat correlates with the function of NK cells that is prominent during the early days of infection. After day 3, the percentage of CD69 is decline steadily and reaching the lowest percentage on the seventh day.

Table 3. Non parametric analysis of CD69 percentage

| Test statistic | percentage |
|---|---|
| D F | 5 |
| Asymp.sig. | .298 |

Based on kruskal-wallis analysis on the percentage of CD69, the p-value is more than $p \geq 0.05$ (p value=0.298). This indicates that the differences of percentages based on daily basis was not significant.

Table 4. Significance of CD69 percentage by daily comparison

| Day of fever | P |
|---|---|
| Day 2 – day 3 | 0.711 |
| Day 3 – day 4 | 0.552 |
| Day 4 – day 5 | 0.776 |
| Day 5 – day 6 | 0.340 |
| Day 6 – day 7 | 0.813 |

Table 4 showed the significance of mean of CD69 percentage by daily comparison. When analyzed on day by day comparison, the difference was also not statistically significant ($p>0.05$) for all seven days of fever. From table 2, CD69 expression increased on day 3 (day 2, mean=42.1839± 15.68765 to day 3, mean= 43.7754 ± 18.72666) as shown by the table, but the increase was not significant (p value=0.711). The same goes to the other comparison, which in table 2 showed a steady decline of CD69 expression starting from day 3 up to day 7 but these declines were not significant ($p >0.05$).

**Fluorescence intensity of CD69 expression.**

CD69 fluorescence intensity was obtained from flowcytometric data in order to examine for the differences in the degree of CD69 expression upon activated NK cells. Since CD69 percentage showed a non normal distribution pattern, median of CD69 expression was used instead of mean.

Table 5. Mean value, minimum and maximum value of CD69 median

| Day | N | Mean+SD | Minimum | Maximum |
|---|---|---|---|---|
| Day 2 | 31 | 30.60±11.12 | 14.07 | 65.52 |
| Day 3 | 35 | 28.37±9.19 | 14.59 | 52.30 |
| Day 4 | 35 | 28.79±9.74 | 14.52 | 48.04 |
| Day 5 | 35 | 31.29±10.86 | 13.82 | 55.48 |
| Day 6 | 34 | 27.42±7.09 | 15.96 | 44.91 |
| Day 7 | 34 | 27.96±8.64 | 14.76 | 48.26 |
| Total | 204 | 29.07±9.44 | 13.82 | 65.52 |

Table 5 shows sample size, mean value, minimum value and maximum value of CD69 median. The mean value have shown considerably a greater activation of NK cells in the fifth day of fever (mean=31.29±10.86). It is not possible to conclude that CD69 expression on NK cells was more dense in fifth day compare to any other day. Also, the intensity of CD69 was found to be higher during early days of fever (day 2 to day 5) as compared to the end of acute phase of fever (day 6 to day 7).

Figure 5 shows that the median of day 3 is slightly lower compared to day 2. However after day 3, the median of CD69 increased up to day 5. This is continued by decrease of the median value on the next day 6 and day 7. The significance of median for this research is to investigate the relationship the intensity of CD69

expression based on the days. The result of Day 5 demonstrated the highest value of median which shows that the highest intensity of CD69 can be found on the fifth day of fever but this value will decrease in the next day after that.

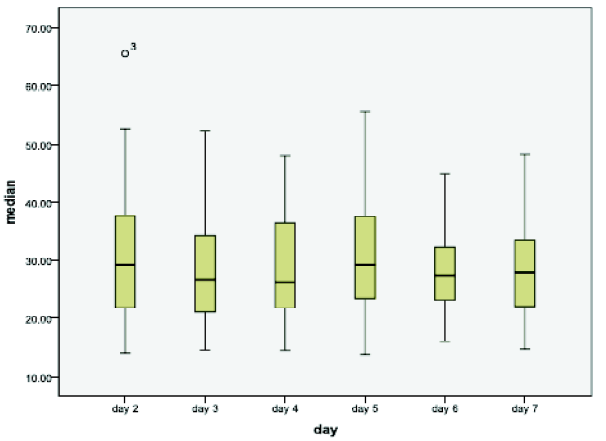


Figure 5. Distribution of median of CD69 based on days of fever

Table 6. Non parametric analysis of CD69 median.

| Test statistic | median |
|---|---|
| Df | 7 |
| Asymp.sig. | 0.532 |

Based on kruskal-wallis analysis on the median of CD69, the p-value is more than $p \geq 0.05$ (p value = 0.532). This indicates that fluorescence intensity of CD69 expression was higher during early days of fever (day 2 to day 5) and being highest on the fifth day (mean = 31.29±10.86), but lower intensity was found during the later days of acute phase (day 6 to day 7), however these differences of CD69 fluorescence intensity was not significant.

The result on kinetics of CD69 expression on activated NK cells was in agreement with Green et al. (1999) and Azeredo et al. (2006) but statistically it was insignificant. For Green et.al that used the Thais children subjects, the study found that the heightened activated NK cells (CD69) were associated with the days of fever as the number of NK cells expressing CD69 is continuously increasing during the first 3 days and drop after the acute phase of fever.

It was reported that the NK cells rates rise during the acute phase of dengue infection[15]. It was also observed that a significant increase in the CD69+ cell percentage among NK cells in dengue patients at early acute phase (days 1-5 with 29.2 ± 13.3%). This increase is maintained at days 6-10 (23.9 ± 17.7%), decreasing after 11 days (13.2 ± 5.0%).

NK cells can be stimulated by direct engagement of activating receptors on the NK cell surface with ligands that are expressed on the target cell surface, so they are poised to respond rapidly to infection[19]. The findings in this study has been supported by other previous research. Apparently, during early phase of infection, the NK cell activity was significantly higher compared to the later phase of dengue infection as it plays an important role as a non adaptive immune response[17,18]. The activation of NK cells induces the expression of several surface markers such as HLA-DP, DQ, DR[20,21], CD38 and also CD69[22] on the cell surface. Such as in dengue infection, the majority of NK cells from dengue infection displayes early markers for activation (CD69, HLA-DR, and CD38) and cell adhesion molecules (CD44, CD11a) during the acute phase of the disease[15,16].

Fluorescence intensity of CD69 showed a pattern that somewhat correlates with what is expected in the acute phase immunopathology

response in infection setting. The intensity of CD69 expression was higher during the early days of fever (day 2 to day 5) and the fifth day of fever showed the highest concentration of CD69 expression. But, the intensity then decreased towards the end of acute phase of infection (day 6 to day 7) that was shown by a lower concentration of CD69 expression. However, these differences were statistically insignificant. This showed that NK cells may have a responsible as immediate innate immune response during acute phase of infection.

The expression of the activation marker CD69 on NK cells stimulated by infection are significantly increased when compared to unstimulated NK cells[23]. Furthermore, during acute phase of dengue infection(day 0 to day 7), it was observed that, even when a dengue infected individual have a lower of NK cells level than non dengue infected individuals, the activated NK cells expressing CD69 was higher in dengue patients compared than non dengue group[24].

There were some limitations in this study when compared to other similar researches. Several confounding factors such as the age of the subjects, race, the presence of other undiagnosed viral infection, and immunization may contribute to the outcome of this research. The sample size was smaller which were taken from 35 patients compared to (51 patients) and (55 patients)[15,16]. Thus it may contribute to a lesser significance in the result of this study. As for the duration of days of dengue infection observed also was shorter. In this research the analysis started on the second day until the seventh day of fever. Conducted observation on acute phase of fever (day 1- day 7) then the patient was followed up until the second week of fever to focus on the kinetics of CD expression on NK cells during convalescent period and compared it with acute phase of infection[15]. There was also no control group made in this study that could to know the differences of NK cells frequency between healthy individuals and dengue patients as conducted[15].

## CONCLUSION

Our result indicates that the expression of activated NK cells is higher during early days of fever then gradually declined towards the end of acute phase of dengue infection. However, there was no statistical significance in kinetics of activated NK cells (CD69) relative count and days of dengue infection.

## SUGGESTIONS

Further researches need to be conducted to understand more about the kinetics of CD69 expression on activated NK cells with suggestion to include control group in the study to function as the standard in comparison to the dengue infection group. A longer observation with follow up of several days after the acute phase of dengue infection may also give a better significant result which we can see when is the exact time the number of CD69 expression on activated NK cells increase steeply, maintained and when it is decrease. The sample size may also need to be bigger to increase the precision that leads to more sensitive hypothesis tests with greater statistical power and smaller confidence intervals.

## REFERENCES

1. WHO. Prevention and control of dengue and dengue hemorrhagic fever: comprehensive

guidelines. WHO Regional Publication, SEARO, 1999.

2. Gubler DJ, Dengue and Dengue Hemorrhagic Fever. Clinical Microbiology Reviews, July, 1998;11(3):480-96.
3. Porter KR, Beckett CG, Kosasih H, Tan RI, Alisjahbana B, Rudiman PIF, Widjaja S, Listiyaningsih E, Ma'roef CN, Wuryadi S. Epidemiology of dengue and dengue hemorrhagic fever in a cohort of adults living in Bandung, West Java, Indonesia. Am. J. Trop. Med. Hyg, 2005;72(1):60-6.
4. Sumarmo. Dengue haemorrhagic fever in Indonesia. Southeast Asian J Trop Med Public Health, 1987;18(3):269-74.
5. Gubler DJ, Suharyono W, Lubis I, Eram S, Sulianti Saroso J. Epidemic dengue hemorrhagic fever in rural Indonesia. Virological and epidemio-logical studies. Am J Trop Med Hyg, 1979;28: 701-10.
6. Eram S, Setyabudi Y, Sadono TI, Sutrisno DS, Gubler DJ Sulianti Saroso J. Epidemic dengue hemorrhagic fever in rural Indonesia. Clinical studies. Am J Trop Med Hyg, 1979;28:711–16.
7. Smith AW, Schwartz E, Current concepts dengue in travelers. N Engl J Med, 2005;353: 924-32.
8. Corwin AL, Larasati RP, Bangs MJ, Wuryadi S, Arjoso S, Sukri N, Listyaningsih E, Hartati S, Namursa R, Anwar Z. Epidemic dengue transmission in southern Sumatra, Indonesia. Trans R Soc Trop Med Hyg May-Jun, 2001,95: 257–265.
9. WHO. Dengue: dengue hemorrhagic fever and dengue shock syndrome in the context of the integrated management of childhood. Geneva, WHO Department of Child and Adolescent Health and Development, 2005.
10. WHO. Dengue: guidelines for diagnosis, treatment, prevention and control — New edition. Geneva, World Health Organization, 2009.
11. Endy TP, Nisalak A, Chunsuttiwat S, Libraty DH, Green S, Rothman AL, Vaughn DW, Ennis FA, 2002. Spatial and temporal circulation of dengue virus serotypes: a prospective study of primary school children in Thailand. Am J.Epidemiol, 2002;156: 52-9.
12. Loke H. Bethel DB, Phuong CXT, Dung M, Scheider J., White NJ, Day PN, Farrar J., Hill V.S. Strong HLA class I-restricted T cell responses in Dengue Hemorrhagic Fever. A double sword? J. Infec Dis, 2001;184:1369-73.
13. Mathew A, Kurane I, Green S, Vaughn DW, Kalayanarooj S, Suntayakorn S, Ennis FA, Rothman AL. Impaired T cell proliferation in acute dengue infection. J Immunol, 1999;162: 5609–15.
14. Vaughn DW, Green S, Kalayanarooj S, Innis BL, Nimmannitya S, Suntayakorn S, Rothman AL, Ennis FA, Nisalak A.Dengue in the early febrile phase: viremia and antibody responses. J Infect Dis, 1997;176:322-30.
15. Azeredo EL, De Oliveira-Pinto LM, Zagne SM, Cerqueira DI, Nogueira RM, Kubelka CF. NK Cells, Displaying Early Activation, Cyto-toxycity and Adhesion Molecules, are Associated with Mild Dengue Disease. Clin Exp Immunol, 2006; 143(2):345-56.
16. Green S, Pichyangkul S, Vaughn DW, et al. Early CD69 expression on peripheral blood lympho-cytes from children with dengue hemorrhagic fever. J Infect Dis, 1999; 180: 1429–35.

17. Homchampa P, S. Sarasombath, et al. Natural killer cells in dengue hemorrhagic fever/dengueshock syndrome. Asian. Pac. J. Allergy Immunol, 1988;6:95-102.
18. Delves PJ, Roitt IM. The immune system. Second of two parts. N Engl J Med, 2000;343: 108-17.
19. Lodoen MB, Lanier LL.Viral modulation of NK cell immunity. Nat Rev Microbiol, 2005;3:59-69.
20. Lima M, Almeida J, dos Anjos Teixeira M, Queiros ML, Justica B, Orfao A. The 'ex vivo' patterns of CD57/CD11c, CD38/CD11b, CD45RA/CD45RO and CD11a/HLA-DR expression identify acute/early and chronic/ late NK-cell activation states. Blood Cells Mol Dis, 2002;28:181-90.
21. Lalezari JP, Beal JA, Ruane PJ, et.al. Low-dose daily subcutaneous interleukin-2 in combination with highly active antiretroviral therapy in HIV+ patients: a randomized controlled trial. HIV Clin Trials, 2000;1:1-15.
22. Rabinowich H, Sedlmayr P, Herberman RB, Whiteside TL. Response of human NK cells to IL-6 alterations of the cell surface phenotype, adhesion to fibronectin and laminin, and tumor necrosis factor-alpha/ beta secretion. J.Immunol, 1993;150:4844-55.
23. Esin S, Batoni G, Counoupas C, Stringaro A, Brancatisano F.L. Direct Binding of Human NK Cell Natural Cytotoxicity Receptor NKp44 to the Surfaces of Mycobacteris. Infection and Immunity, 2008;76:1719-27.
24. Dietze R. Research and Training in Tropical and Emerging Diseases in Brazil. U.S Army Medical Research and Material Command, 2000.