

INDONESIAN JOURNAL OF  
**CLINICAL PATHOLOGY AND  
MEDICAL LABORATORY**

Majalah Patologi Klinik Indonesia dan Laboratorium Medik

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Website: <http://www.indonesianjournalofclinicalpathology.or.id>

**Accredited No. 36a/E/KPT/2016, Tanggal 23 Mei 2016**

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**Thanks to editors in duty of IJCP & ML Vol 23 No. 1 November 2016**

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Adi Koesoema Aman, Edi Widjajanto, AAG. Sudewa, Nurhayana Sennang AN

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RESEARCH

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## THE CORRELATION OF NAIVE CD4<sup>+</sup>T LYMPHOCYTE CELL PERCENTAGE, INTERLEUKIN-4 LEVELS AND TOTAL IMMUNOGLOBULIN E IN PATIENTS WITH ALLERGIC ASTHMA

(Kenasaban antara Persentase Sel Limfosit T-CD4<sup>+</sup> Naive dengan Kadar Interleukin-4 dan Jumlah Imunoglobulin E Total di Pasien Asma Alergi)

Si Ngr. Oka Putrawan<sup>1</sup>, Endang Retnowati<sup>1</sup>, Daniel Maranatha<sup>2</sup>

### ABSTRAK

Asma alergi merupakan fenotip asma paling sering didapatkan di anak dan dewasa. Allergen masuk ke saluran napas yang akan memicu respons imun sehingga menimbulkan gejala asma. Tujuan penelitian ini adalah membuktikan adanya kenasaban antara persentase sel limfosit T-CD4<sup>+</sup> naive dengan kadar IL-4 dan jumlah IgE total di pasien asma alergi. Penelitian bersifat analitik observasional dengan rancangan potong lintang. Sampel terdiri dari 25 pasien asma alergi yang diperiksa di Ruang Poliklinik Asma dan Poliklinik Paru RSUD Dr. Soetomo. Pemeriksaan persentase sel limfosit T-CD4<sup>+</sup> naive dengan flowcytometri, kadar IL-4 dengan Enzyme Linked Immunosorbent Assay (ELISA), dan jumlah IgE Total dengan chemiluminescence. Persentase sel limfosit T-CD4<sup>+</sup> naive cenderung meningkat yang berkisar antara 25,74–47,68% dengan rerata 36,72% dan Simpang Baku (SB) 6,0%. Kadar IL4 meningkat berkisar antara 43,4–97,2 pg/mL dengan rerata 70,8 pg/mL dan SD 14,95 pg/mL. Jumlah IgE total juga meningkat berkisar antara 231,8–684,8 IU/mL dengan rerata 410,9 IU/mL dan SD 114,65 IU/mL. Terdapat kenasaban antara persentase sel limfosit T-CD4<sup>+</sup> naive dengan kadar IL-4 dan jumlah IgE total di pasien asma alergi.

**Kata kunci:** Sel limfosit T-CD4<sup>+</sup> naive, IL-4, IgE total, pasien asma alergi

### ABSTRACT

Allergic asthma is the most common asthma phenotype found in children and adults. Allergen will enter the respiratory tract and trigger an immune response, causing asthma symptoms. The aim this study was to prove the existence of a correlation between the percentage of naive CD4<sup>+</sup> T cell lymphocytes with levels of IL-4 and the amount of total IgE in patients with allergic asthma. This was analytic observational study with cross sectional design. Samples consisted of 25 patients with allergic asthma. Examination of the percentage of naive CD4<sup>+</sup> T cell lymphocytes was done by flowcytometry, levels of IL-4 was determined by Enzyme Linked Immunosorbent Assay (ELISA) and amount of total IgE was by chemiluminescence. The percentage of naive CD4<sup>+</sup> T cell lymphocytes was likely to increase in the range of 25.74 to 47.68% with a mean of 36.72% and a standard deviation (SD) of 6.0%. Levels of IL-4 ranged from 43.4 to 97.2 pg/mL with a mean of 70.8 pg/mL and SD of 14.9 pg/mL. The amount of total IgE also increase and ranged from 231.8 to 684.8 IU/mL with a mean of 410.9 IU/mL and SD of 114.6 IU/mL. There was a correlation between the percentage of naive CD4<sup>+</sup> T cell lymphocytes with levels of IL-4 and the amount of total IgE in patients with allergic asthma.

**Key words:** Naive CD4<sup>+</sup> T lymphocytes, IL-4, total IgE, allergic asthma patients

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### INTRODUCTION

Asthma is a chronic respiratory disease considered as a serious public health problem in many countries around the world. Asthma can be a light one that

does not interfere daily activity, or moderate and severe ones that interfere daily activity. Patients may also experience disability, leading to a decline in productivity and life quality.<sup>1</sup>

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Based on data of Household Health Survey (Survey Kesehatan Rumah Tangga-SKRT) in various provinces in Indonesia, asthma is on the fifth position out of the top ten as a cause of morbidity together with chronic bronchitis and emphysema. Asthma, chronic bronchitis, and emphysema even are in the fourth position as causes of death (mortality) in Indonesia (5.6%). Data of Household Health Survey also report that the prevalence of asthma throughout Indonesia is at 13/1,000 people.<sup>1</sup> Allergic asthma, moreover, is phenotype asthma mostly found in children and adults. The prevalence of allergic asthma is reported between 45% to 88% of all asthma patients, whereas in adults is estimated between 60% and 75%.<sup>2</sup>

The immune response begins with the movement of allergens into the respiratory, then captured by dendritic cells, *Antigen Presenting Cells* (APC). Next, the antigens are processed within APC and presented via *Major Histocompatibility Complex* (MHC) to *naive CD4<sup>+</sup> T lymphocytes* in peripheral, then leading to activation of cytokine synthesis and secretion. Cytokine is a growth and differentiation factor causing differentiation of *naive CD4<sup>+</sup> T lymphocytes* into *T helper 2 (T-h2)*. The differentiation of *naive CD4<sup>+</sup> T lymphocytes* into *Th2 cells* is affected by microenvironment, such as *interleukin (IL)-4*, *IL-2*, and *IFN-γ*.<sup>3</sup> *IL-4* in the microenvironment is a strong stimulation of *naive CD4<sup>+</sup> T lymphocytes* into *Th2 cells*. *Naive CD4<sup>+</sup> T lymphocytes* can produce *IL-4* since the beginning of the stimulation.<sup>3</sup>

The stimulation of *IL-4* and *IL-13* from *Th2 cells* then will stimulate the *B lymphocytes* to synthesize *IgE*. Next, *IgE* will be secreted by *B lymphocytes*, and attached to the surface of *mast cells*. If the same allergens move into again, they will be bound by *IgE* on the surface of *mast cells*. *IgE* activates *mast cells* causing *mast cell degranulation* and resulting in the release of mediators that can cause asthma symptoms.<sup>3</sup> Therefore, this research aimed to prove the correlation of *naive CD4<sup>+</sup> T lymphocytes*, *IL-4* levels and total *IgE* in patients with allergic asthma.

## METHODS

This research was an analytic observational study with cross sectional design. This research was conducted at Polylinic Section for Asthma, Polylinic Section for Lung, and Clinical Pathology-Installation Department of Faculty of Medicine, Airlangga University-Dr. Soetomo Hospital from May to October 2015. The subjects of this research were patients with

allergic asthma visiting Polylinic Section for Asthma and for Lung in Dr. Soetomo Hospital. The number of the subjects obtained was 25 people.

Next, samples were taken from patients with allergic asthma. There were some inclusion criteria. First, patients had to be diagnosed with allergic asthma by doctors in Polylinic Section for Asthma and for Lung in Dr. Soetomo Hospital. Second, patients had to be in the age of ≥21 years old. Third, patients had to be willing to participate in this research and then signed informed consent. On the other hand, there were also some exclusion criteria. First, patients were diagnosed with non-atopic asthma. Second, patients were also diagnosed with other lung diseases or systemic one. Third, patients with asthma received corticosteroid treatment or antihistamines less than one week before the samples were taken.

In addition, patients with asthma taken as the subjects of this research had to be patients with allergic asthma by history of allergies, history of allergies in their family, or history of asthma in their family. Asthma symptoms occur when exposed to allergens, such as dust mites, animal dander, or food. Next, the percentage of *naive CD4<sup>+</sup> T lymphocytes* was measured based on examination results using *flowcytometri FACS Calibur* method. Serum *IL-4* levels then was measured based on examination results of *IL-4* levels in the serum of patients with allergic asthma using *Enzyme Linked Immuno Sorbent Assay* method (ELISA). Total *IgE* in the serum of patients with allergic asthma was measured using *chemiluminescence* method with a *two-site sandwich immunoassay* principle.

Next, the percentage of *naive CD4<sup>+</sup> T lymphocytes* was examined using whole blood samples. On the other hand, *IL-4* levels and total *IgE* were examined using serum samples. The examination of the percentage of *naive CD4<sup>+</sup> T lymphocytes* was conducted using flowcytometry. This examination also used *FACS Calibur* instrument from Becton Dickinson (BD) with *BD PharMingen™ Human Naive/Memory T Cell Panel* reagents.<sup>4</sup>

The principle of the examination, moreover, was adding 100 uL of blood samples (whole blood) into the reagents. As a result, the antibody labeled with *fluorochrome* at the reagents bound specifically to the antigens on the surface of lymphocytes. Lymphocytes binding to the antibodies that had been marked with *fluorochrome* then would fluoresce when exposed to laser light. Next, *naive CD4<sup>+</sup> T lymphocytes* were characterized by positive *CD45RA* and *CD197 AF647*. Fluorescence that occurs is in proportion to the percentage of cells existed.<sup>5</sup>

Method used for the examination of IL-4, furthermore, was *Enzyme Linked Immuno Sorben Assay* (ELISA) using *E Bioscience Human IL-4 Platinum ELISA*. IL-4 in the serum would bind to anti-human IL-4 monoclonal antibody, which had been attached to the wells. Antibodies that were not bound were removed through washing. Next, the complex monoclonal anti-human IL-4 antibody then was bound with conjugated biotin anti-human IL-4 antibody added, and then added with streptavidin-HRP. Thus, an antibody-antigen-antibody, complex “sandwich” was formed. The substrate solution then was reactive with HRP added, generating the appropriate color (proportional) to the amount of IL-4 contained in the samples. Reading the results then was performed at a wavelength of 450 nm.<sup>6</sup>

Examination of total IgE then was quantitatively conducted *in vitro* method using Chemiluminescence and *ADVIA Centaur* instruments as well as *ADVIA Centaur XP* system. The principle of this method is a two-site sandwich immunoassay using direct chemiluminometric technology with two antibodies against IgE in a certain amount. The first antibody contained in Lite reagents was *goat anti-human IgE antibody* labeled with acridinium ester. The second antibody covalently bound to a paramagnetic particle was *mouse anti-human IgE antibody*. Results of Chemiluminescence reaction then were read with luminometer and reported in a unit of IU/mL. Next, the number of relative light units (RLU) detected by the system was proportional to the amount of total IgE contained in the serum of patients. Reference normal value for serum total IgE is 150–300 IU/mL.<sup>7</sup> Finally, a statistical test, Pearson correlation test, was conducted to analyze the correlation of the percentage of *naive CD4<sup>+</sup> T lymphocytes*, IL-4 levels, and total IgE in patients with allergic asthma.

## RESULTS AND DISCUSSION

This research was conducted from May to October 2015. The number of samples that met the inclusion criteria was 25 people. The mean of the patients' age with allergic asthma in this research was 40.24 years old, ( $SD \pm 11.42$ ) years old with the lifespan of between 21–67 years. The number of male samples was 11 people (44%), while the number of female samples was 14 (56%).

Those patients mostly experiencing allergic asthma were in the age range of 31–41 years old, while the life span of those patients suffering from allergic asthma at least was at the age of 61–70 years.

**Table 1.** Characteristics of research subjects

Characteristics of samples	Number	Percentage (%)
Age (Mean $\pm$ SD)	40.24 $\pm$ 11.42 tahun	
Sex		
Males	11	44%
Females	14	56%

**Table 2.** Distribution of patients by age

Age (years old)	Number	Percentage (%)
21–30	6	24.0
31–40	8	32.0
41–50	5	20.0
51–60	5	20.0
61–70	1	4.0
Total	25	100.0

According to studies conducted by the *Australian Institute of Health and Welfare* (2007), the incidence of asthma in the age group of 18–34 years is 14%, while in the age group of >65 years decreases to 8.8%. In Jakarta, a study at RSUP Persahabatan shows that the average incidence of asthma is in the age of 46 years old.<sup>1</sup>

The mean age of the patients with asthma in this research was similar to the results in the previous research conducted by the Australian Institute of Health and Welfare. The incidence of allergic asthma increased at the age of 42 years because in this age range can be considered as a productive age, so often exposed to allergens.

The proportion of the sexes in the research sample was 11 male patients (44%) and 15 female patients (56%). According to GINA (2009) and the NHLBI (2007), male children have a higher risk factor for asthma. The prevalence ratio on puberty shifts and becomes this risk more common in female. However, there is no difference in the incidence of asthma in adulthood between the sexes. Hormonal factors in women are usually suspected of causing fluctuation in the incidence of allergic asthma, but until now, it still has not proven.<sup>8</sup>

The percentage of *naive CD4<sup>+</sup> T lymphocytes* in 25 patients with allergic asthma in this research, furthermore, ranged from 25.74 to 47.68% with the average of 36.72% and a standard deviation of 6.0%. It means that the results of this research show an increase in the percentage of *naive CD4<sup>+</sup> T lymphocytes*, while the normal percentage of *naive CD4<sup>+</sup> T lymphocytes*

is between 25.4–33.8%. The increase was due to the activation of naive CD4<sup>+</sup> T lymphocytes after their interaction with antigen-MHC II and then their differentiation into specific subtypes depending mainly on the microenvironment. Differentiation of naive CD4<sup>+</sup> T lymphocytes is in accordance with the signals they receive during the initial interaction with antigens.<sup>9,10</sup>

The early presence of IL-4 in micro-environment can actually be considered as a strong impulse to evolve toward Th2 cells. The mechanism responsible for the initial production of IL-4 and IL-4 source, however, is still unknown. But, the naive CD4<sup>+</sup> T lymphocytes themselves can produce small amounts of IL-4 since the beginning of stimulation. Effects of IL-4 induction dominate other cytokines, as a result, when the levels of IL-4 reach the point required, Th cells will differentiate into Th2.<sup>11</sup>

In addition, the results of this research show that the levels of IL-4 in 25 patients with allergic asthma ranged from 43.40 to 97.20 pg/mL with a mean of 70.77 pg/mL and a Standard Deviation (SD) of 14.95 pg/mL. The results indicate that IL-4 levels increase in patients with allergic asthma compared to those in a healthy population, that is about less than 0.4 pg/mL.

Similarly, a research conducted by Kraan *et al*<sup>12</sup> show an increase in IL-4 levels in patients with allergic asthma compared to patients with non-allergic asthma. In other words, there is a strong positive correlation between IL-4 levels and the amount of IgE in patients with allergic asthma.<sup>12</sup> Another research conducted by Afshari *et al*<sup>12</sup> in patients with allergic asthma also shows a significant increase in IL-4 levels in patients with allergic asthma.

Levels of IL-4 in patients with allergic asthma can increase since IL-4 itself has been there since the beginning of the microenvironment, considered as a strong stimulus for naive CD4<sup>+</sup> T-cell lymphocytes to evolve toward Th2 cells. The primary mechanism for the production of IL-4 and IL-4 source, however, is still unknown. But, naive CD4<sup>+</sup> T-cell lymphocytes themselves can produce small amounts of IL-4 since the beginning of stimulation. Effects of IL-4 induction dominate other cytokines, as a result, when the levels of IL-4 reach the point required, Th cells will differentiate into Th2.<sup>3</sup>

High total IgE actually often help diagnosis. Total IgE in patients with allergies usually fluctuates based on the individual's exposure to allergens. Sensitivity test ranges between 60–95%, while specificity ranges between 30–95%. The amount of total IgE is generally higher in adult individuals and children who are atopic than in the non-atopic ones at the same age.

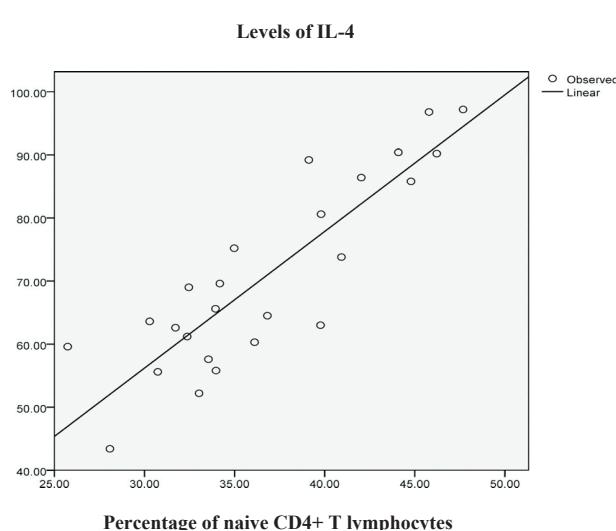
Allergic responses, therefore, depend on the amount and specificity of IgE to allergens. Serum IgE generally increases with age until the age of 10 years and then decreases as the increasing of the age, hence, examination of total IgE is important in accordance with the age pattern of a person in order to define whether someone is allergic or not. Measurement of total IgE in adults is also useful when the number of total serum IgE is >100 IU/mL.

The amount of total IgE in patients with allergic asthma in this research ranged from 231.80 to 684.80 IU/mL with a mean of 410.98 IU/mL and a standard deviation (SD) of 114.65 IU/mL. Meanwhile, referenced normal values for serum total IgE is 150–300 IU/mL. It means that the results in this research show an increase in total IgE. Similarly, a research conducted by Kraan *et al*<sup>12</sup> shows that there is a meaningful increase in IgE in patients with allergic asthma.

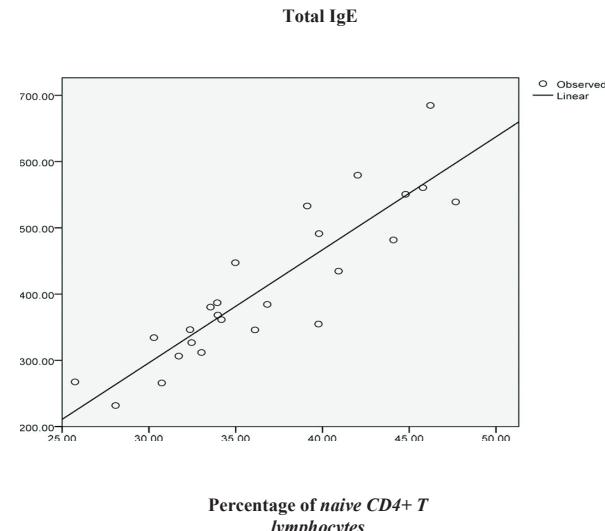
The results are also consistent with a research conducted by Boyce *et al*<sup>8</sup> The research was conducted in patients with allergic asthma by examining the amount of total IgE and IgE increasing significantly in patients with allergic asthma. The study also show that IgE is responsible for allergic reactions and the development of respiratory tract inflammation in patients with allergic asthma.

Another research conducted by Holgate *et al*<sup>8</sup> shows that the development of monoclonal antibodies against IgE may reduce the amount of total IgE. Thus, it can be used as an effective treatment for allergic asthma.

The increasing amount of total IgE in patients with allergic asthma, moreover, can occur because when the patients are exposed to allergens, it will cause activation of Th2 cells. Th2 cells are activated to produce inflammatory cytokines, such as IL-4 and IL-3, which stimulate B cells to proliferate and produce IgE. IgE secreted by B lymphocytes will attach to *high affiniting IgE receptors* (FcεRI) on the surface of mast cells. If the same allergens enter and then it will be bound by the IgE on the surface of mast cells, *Cross linked receptor* of IgE with allergens then will activate mast cells that causes mast cell degranulation resulting in the release of mediators, such as histamine, prostaglandins, leukotrienes that cause bronchial smooth muscle contraction, mucus secretion and vasodilatation. Next, inflammatory mediators induce microvascular leak involving exudation of plasma into the respiratory tract. The leakage of plasma protein then induces thickening and edema of respiratory track walls which cause a narrowing of the lumen of the respiratory tract, triggering respiratory muscle contraction.<sup>2,13</sup>



**Figure 1.** Correlation between the percentage of naive CD4<sup>+</sup> T lymphocytes and levels of IL-4 in patients with allergic asthma.



**Figure 2.** Correlation between the percentage of naive CD4<sup>+</sup> T lymphocytes and total IgE in patients with allergic asthma.

Consequently, the number of Th2 cells that produce IL-4 in the circulation of patients with atopy is much higher than in those with non-atopy. Similarly, the amount of IL-4 secreted is also higher. These conditions can increase the synthesis of IgE in patients with atopy. Besides produced by Th2 cells, IL-4 and IL-13 are also produced by cells that express Fc $\epsilon$ RI, namely basophils and mast cells. The cytokines IL-4 and IL-13 then stimulate enhancement of IgE biosynthesis and changes in naive CD4<sup>+</sup> T-cell lymphocyte cells into Th2 causing increased secretion of cytokines that play a role in inflammation process that occurs in allergy.<sup>3</sup>

Next, data of the percentage of naive CD4<sup>+</sup> T lymphocytes and IL-4 in patients with allergic asthma were analyzed using Pearson correlation test. Pearson correlation test was performed to determine the correlation between the percentage of naive CD4<sup>+</sup> T lymphocytes and IL-4 levels in patients with allergic asthma. The results of the test obtained p value <0.05. It indicates a strong positive correlation between the percentage of naive CD4<sup>+</sup> T lymphocytes and IL-4 levels, with p=0.000 and r=0.870 (p=0.000).

Similar research conducted by Thruth *et al*<sup>12</sup> on mice shows a strong positive correlation between naive CD4<sup>+</sup> T lymphocytes and IL-4. This research also shows that high naive CD4<sup>+</sup> T lymphocytes will increase IL-4. Similarly, an increase in IL-4 will stimulate naive CD4<sup>+</sup> T lymphocytes to Th2 pathway.<sup>12</sup>

Immune response actually begins with the movement of allergens into respiratory tract to be captured by dendritic cells as cells recognizing antigens (*Antigen Presenting Cell/APC*). Antigens then

are processed within APC and presented via *Major Histocompatibility Complex* (MHC) to *naive* CD4<sup>+</sup> T lymphocytes in peripheral, stimulating activation of the synthesis and secretion of cytokines. IL-4 and IL-13 are autocrine growths and differentiation factors triggering *naive* CD4<sup>+</sup> T lymphocytes to differentiate into Th2 phenotype influenced by both genetic and micro environment.<sup>11</sup>

The early presence of IL-4 in the micro environment, furthermore, is a strong impulse to evolve toward Th2 cells. The mechanism responsible for the initial production of IL-4 and IL-4 source, nevertheless, is still unknown. But, *naive* CD4<sup>+</sup> T lymphocytes can produce small amounts of IL-4 since the beginning of stimulation. Effects of IL-4 induction dominate other cytokines, as a result, when the levels of IL-4 reach the point required, Th cells will differentiate into Th2.<sup>11</sup>

IL-4 is the main regulator of Th2 immune response, which may act directly on B cells and induce the formation of IgE. IL-4 also works on naive CD4<sup>+</sup> T lymphocytes to differentiate into Th2 pathway. IL-4 then regulates the formation of IL5. IL5 itself is not very important in Th2 responses, but it can trigger activity and proliferation of eosinophils. Eosinophils are active, and their products in the respiratory tract can cause inflammation and mucus secretion, which are instrumental in the emergence of asthma.<sup>15</sup>

The results of the analysis in patient with allergic asthma also show a strong positive correlation between the percentage of naive CD4<sup>+</sup> T lymphocytes and total IgE (p=0.00) with r=0.757 r=0.757 (p=0.000).

Similarly, the research conducted by Thruth *et al*<sup>12</sup> on mice shows that there is a strong positive correlation between naive CD4<sup>+</sup> T lymphocytes and total IgE due to the stimulation of antigens on naive CD4<sup>+</sup> T lymphocytes causing increased secretion of IL-4. IL-4 then stimulating B cells to produce.

The correlation between asthma and total IgE actually has already known in previous researches. IgE in patients with allergic asthma is both in serum and on the surface of mast cells. IgE level in patients with allergic asthma is higher than in non-allergic individuals. Increased levels of IgE, thus, is an important indicator for the diagnosis of allergic asthma.<sup>14</sup>

Activation of naive CD4<sup>+</sup> T lymphocytes, moreover, occurs in secondary lymphoid organs and peripheral circulation where naive CD4<sup>+</sup> T lymphocytes usually circulate, so it possible to interact with antigens presented by APC. Cells then will secrete interleukin IL-4 and IL-13 activating B lymphocytes to secrete IgE.<sup>9, 15</sup>

Finally, the number of Th2 producing IL-4 in the circulation of patients with atopy is actually higher than in those with non-atopy. This condition then will increase the synthesis of IgE in patients with asthma.<sup>15</sup>

## CONCLUSION AND SUGGESTION

Based on the results of this research it can be concluded that the percentage of naive CD4<sup>+</sup> T lymphocytes in patients with allergic asthma in Polylinic Section for Asthma and Polylinic Section for Lung in Dr. Soetomo Hospital increased. The results also showed an increase in IL-4 in those patients with allergic asthma. Similary, total IgE in those patients with allergic increased. Therefore, it can be said there is a strong positive correlation between the percentage of naive CD4<sup>+</sup> T lymphocytes and total IgE in patients with allergic asthma.

Further researches on cytokines IL-9 and IL-13 as well as on specific IgE, consequently, are needed to determine the correlation of naive CD4<sup>+</sup> T lymphocytes

and total IgE in patients with allergic asthma. Further researches also had better involve a broader population of patients with allergic asthma to obtain consistency of results.

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