

## Neopterin as Immune Marker in HIV infection การใช้นีออปเทอรินเป็นดัชนีภูมิคุ้มกันสำหรับผู้ติดเชื้อเอชไอวี

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ศึกษาระดับนีออปเทอรินในซีรัมผู้ติดเชื้อและไม่ติดเชื้อเอชไอวี วัดระดับนีออปเทอรินในซีรัมด้วยวิธี ELISA โดยใช้ชุดน้ำยาสำเร็จรูป พบว่าค่าเฉลี่ย  $\pm$  ส่วนเบี่ยงเบนมาตรฐาน ของกลุ่มอ้างอิง (กลุ่มไม่ติดเชื้อเอชไอวี 122 คน) เท่ากับ  $1.4 \pm 0.93$  นาโนกรัม/มิลลิลิตร ผู้ติดเชื้อเอชไอวีมีระดับนีออปเทอรินสูงขึ้นอย่างมีนัยสำคัญ คือเท่ากับ  $3.8 \pm 2.94$  นาโนกรัม/มิลลิลิตร (กลุ่มติดเชื้อเอชไอวี 35 คน) ช่วงความเชื่อมั่นของค่าเฉลี่ย ร้อยละ 95 อยู่ระหว่าง 1.2 ถึง 1.6 นาโนกรัม/มิลลิลิตร ในกลุ่มอ้างอิงและอยู่ระหว่าง 2.8 ถึง 4.8 นาโนกรัม/มิลลิลิตร ในกลุ่มติดเชื้อ ระดับนีออปเทอรินที่เพิ่มขึ้นมีความสัมพันธ์อย่างมีนัยสำคัญกับจำนวนซีดี4 ที่ลดลง ( $r = -0.485, p=0.000$ ) การใช้นีออปเทอรินช่วยพยากรณ์หรือประเมินอาการป่วยจากการติดเชื้อเอชไอวี น่าจะเป็นประโยชน์สำหรับพื้นที่ที่ไม่สามารถตรวจวัดจำนวนซีดี4 หรือจำนวนไวรัสได้

**คำสำคัญ :** นีออปเทอริน ดัชนีภูมิคุ้มกัน การติดเชื้อเอชไอวี

**Abstract :** Chanida Palanuvej. 2001. Neopterin as immune marker in HIV infection. Thai J Hlth Resch 15(2) : 131-138.

Serum neopterin concentration was studied in HIV-infected and non-infected Thai. Measurement of neopterin in serum by commercial ELISA test kit resulted the reference mean  $\pm$  SD of  $1.4 \pm 0.93$  ng/ml (from 122 HIV-seronegatives). Infection with HIV significantly elevated neopterin concentration to mean  $\pm$  SD of  $3.8 \pm 2.94$  ng/ml (from 35 HIV-seropositives). The 95% CI for mean among two groups were 1.2,1.6 ng/ml and 2.8,4.8 ng/ml respectively. Increase of neopterin concentration significantly correlated with decrease of CD4 cell number ( $r = -0.485, p=0.000$ ). The use of neopterin to prognosticate or assessing HIV related clinical outcome had advantage where measurements of CD4 cell and viral load are not available.

**Key words :** neopterin, immune marker, HIV infection

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## Introduction

Neopterin is synthesized *in vivo* by interferon- $\gamma$  activated monocytes and macrophages. Biosynthetic pathway of neopterin is shown in figure 1. Guanosine triphosphate (GTP) is converted to 7,8-dihydroneopterin triphosphate ( $\text{NH}_2\text{TP}$ ), 7,8-dihydroneopterin ( $\text{NH}_2$ ) and neopterin respectively. In liver and neuroendocrine tissues,  $\text{NH}_2\text{TP}$  is converted to 5,6,7,8-tetrahydrobiopterin ( $\text{BH}_4$ ) instead of  $\text{NH}_2$ .  $\text{BH}_4$  is an electron donor which involves in catecholamines or serotonin synthesis via the hydroxylation of phenylalanine to tyrosine, tyrosine to l-dopa and tryptophan to 5-hydroxytryptophan (Muller *et al.*,1991).

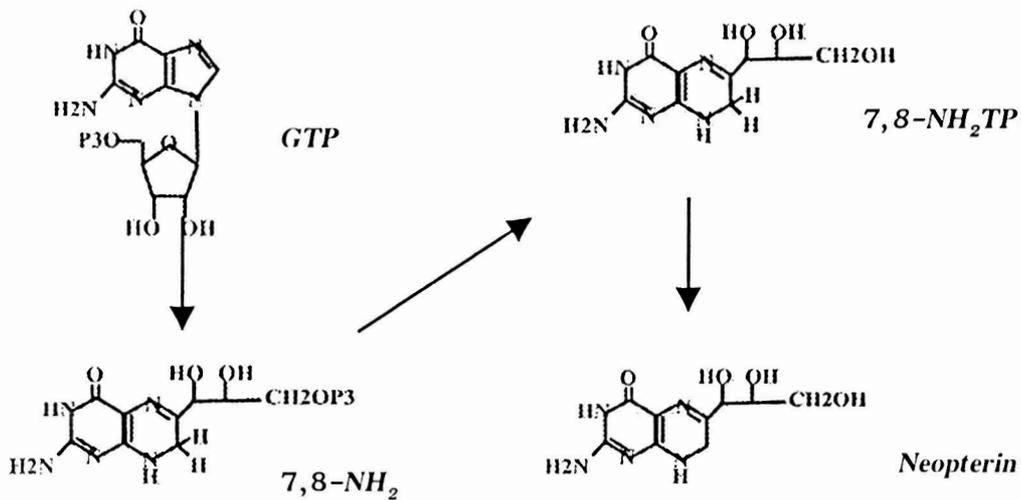


Figure 1 Biosynthesis of neopterin

During infection, activated cell-mediated immunity leads to neopterin secretion. So neopterin concentration in serum and urine specimen can be used as marker of immune stimulation in many infectious diseases including HIV infection and AIDS. In HIV infection, increase in concentration of neopterin is correlated with progression of the disease, as shown in figure 2 (Baier-Bitterlich *et al.*,1996). Macrophages activation by interferon- $\gamma$  secrete tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and neopterins which may induce HIV-DNA replication (Baier-Bitterlich *et al.*,1996). Serum concentration of neopterin among HIV-infected and non-infected persons was studied for the purpose of neopterin use as an surrogate marker for prognosis of HIV progression in Thai population.

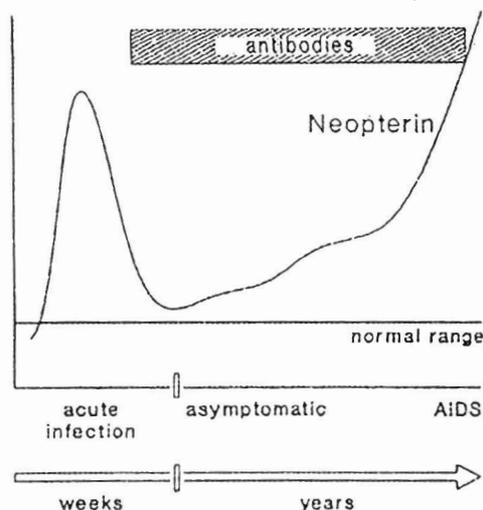


Figure 2 Neopterin concentration during the course of HIV infection (Baier-Bitterlich *et al.*,1996)

### Materials and Methods

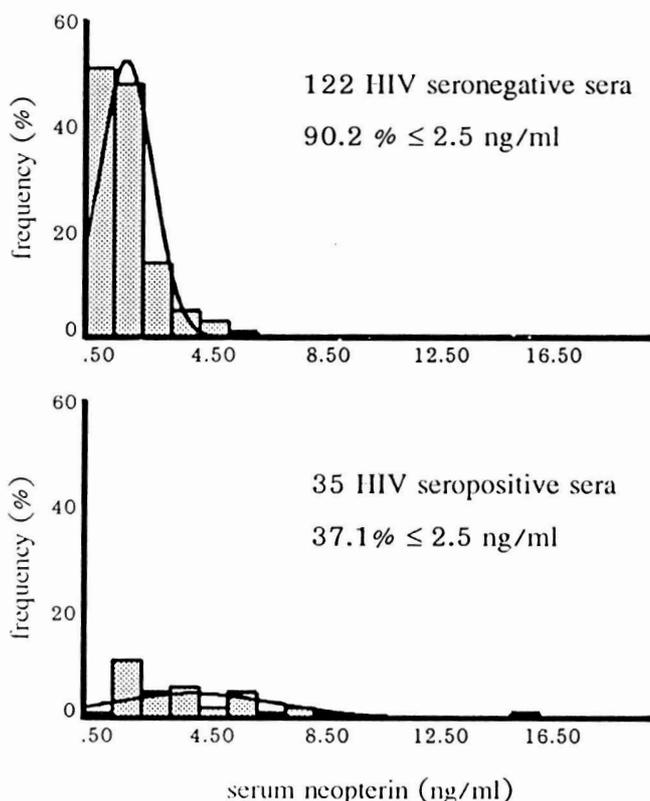
Serum of HIV-seronegatives and HIV-seropositives aged 20-50 years were collected and kept frozen at  $-70^{\circ}\text{C}$  until analysis. Neopterin concentration in serum was measured by enzyme linked immunosorbent assay commercial kit (Immuno Biological Laboratories (IBL), Hamburg, Germany) according to the manufacturer's instruction (Table 1). Each subject was collected for serum twice at 3 months interval. Some subjects' blood was analysed for CD4 cell counts at the same time of serum collection. Neopterin concentration of each subject was average from two collected sera. The concentration between two groups was compared by t-test statistics. Neopterin concentration and CD4 cell counts was compared by correlation statistics.

Table 1 Assay of serum neopterin by commercial ELISA kit

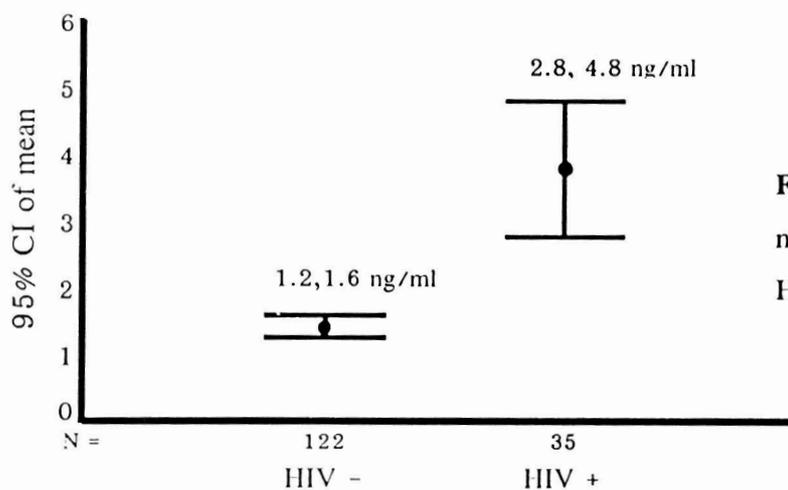
reagents	Volume ( $\mu\text{l}$ )
Standard neopterin, Control or Serum	10
Enzyme conjugate (dilute 1:201 with Assay buffer)	100
Neopterin antiserum	50
<i>Cover the plate with the black adhesive foil and shake in the dark at room temperature for 90 minutes</i>	
<i>Wash the plate 3 times with Wash buffer (dilute 1:20 with distilled water)</i>	
Substrate solution (dilute 1:31 with Substrate buffer)	200
<i>Incubate at room temperature for 10 minutes</i>	
Stop solution (1M $\text{H}_2\text{SO}_4$ )	100
<i>Mix gently and read the optical density at 450 nm with a microtiter plate reader within 60 minutes after stopping the reaction</i>	

**Results**

Neopterin concentration in 122 sera of HIV-seronegatives ranged from 0.5 – 5.6 ng/ml with the mean  $\pm$  SD of  $1.4 \pm 0.93$  ng/ml (Figure 4). HIV infected persons had higher concentration of serum neopterin ( $p=0.000$ ). The concentration in 35 HIV-seropositive sera ranged from 0.6 – 15.7 ng/ml with the mean  $\pm$  SD of  $3.8 \pm 2.94$  ng/ml (Figure 4 and 5)



**Figure 4** Histogram of serum neopterin among HIV seronegatives and HIV seropositives



**Figure 5** 95% CI of mean of serum neopterin among HIV seronegative and HIV seropositive group

The scatter plot between neopterin concentration and CD4 cell counts of 57 pairs of serum was shown in Figure 6. There was relationship between this two markers with the coefficient of correlation of  $-0.485$  ( $p=0.000$ ).

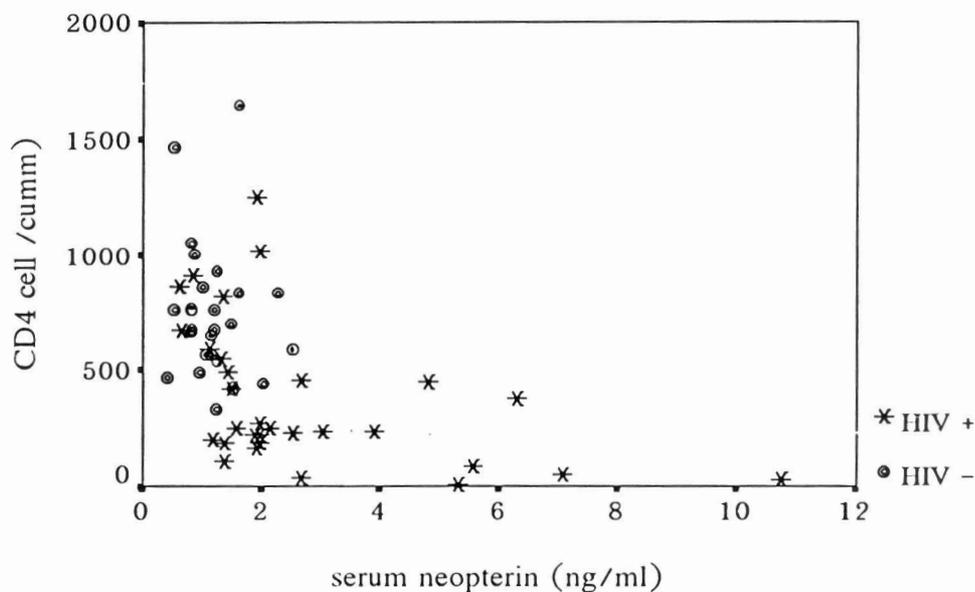


Figure 6 The scatter plot between serum neopterin and CD4 cell counts

### Discussion

Neopterin can be determined directly in serum by commercial ELISA kit. Some precaution should be in mind such as the specimen should not be exposed strongly to light and hemolysed specimen should be avoided. The cost of analysis was about 300 baht per test, which was less expensive than CD4 cell counts assay. The instrument for ELISA technique was less complicated than the flow cytometer and more available in many hospital laboratories.

Neopterin was not sex-dependent different, and slightly increase in age over 75 years (Muller *et al.*,1991). The upper limit of the reference range of serum neopterin was 2.5 ng/ml (10 nmol/L). This value was derived from the 98<sup>th</sup> percentile of the distribution of neopterin concentration in sera from about 80,000 healthy blood donors (Millner *et al.*, 1998). The reference range reported in Muller *et al.*'s review (1991) was higher as 0-3.75 ng/ml (0-15 nmol/l). Serum neopterin in Aziz *et al.* (1999)'s study ranged from

0.775 to 1.5 ng/ml in normal subjects and from 2.8 to 5.0 ng/ml in HIV-positives with median CD4 of 310 cells/cu mm and from 1.6 to 19.4 ng/ml in HIV-positives with median CD4 of 28 cells/cu mm.

Serum neopterin concentration in Thai population from this study (n=122) was  $1.4 \pm 0.93$  ng/ml (ranged from 0.5-5.6 ng/ml). The upper limit which corresponded to the mean+3SD was 4.2 ng/ml. The average of neopterin concentration, from 2 sera at 3 month interval of each subject, was used to reduce variation from spot serum. The control subjects in this study were not recruited on the criteria of healthy or normal persons, but on a circumstance of non-HIV infection. This might lead to higher range of serum neopterin compared to other studies described above. However, these subjects which were the villagers and factory workers represented the immune status among Thai rural people. The median CD4 cell counts among these HIV-seronegatives was 690 cells/cu mm and the range was 340-1650 cells/cu mm. In HIV-infected group, serum neopterin significantly elevated. The 95% confidence interval (CI) for mean between HIV-infected and HIV-noninfected groups were 2.8,4.8 and 1.2,1.6 ng/ml respectively. The median CD4 cell counts among these HIV-seropositives was 245 cells/cu mm and the range was 10-1250 cells/cu mm. The increase of neopterin concentration was significantly correlated with the decrease in CD4 cell counts ( $r=-0.485$ ,  $p=0.000$ ). Neopterin could be useful in Thai population as a surrogate marker for AIDS progression. It's also possible to use for assessment of antiretroviral treatment (Fahey,1998).

The studies later found that increase of neopterin and other soluble products of immune activation occurred before the CD4 cell inflection point and the increase of plasma viral load in HIV infection. There are substantial evidences that immune cell activation up-regulates HIV expression in infected cells (Salazar-Gonzalez.,1998; Nishanian *et al.*, 1998). Combination of immune activation markers with CD4 cell counts improved the prognostic capacity (Fahey *et al.*,1998). In some population such as intravenous drug abuser and infants, neopterin better correlated with HIV prognosis than beta-2-microglobulin (Fahey,1998).

On the other hand, neopterin is nonspecific for HIV progression. It can be elevated in non-AIDS-related diseases such as inherited defects of pterin metabolism (atypical phenylketonuria); cytomegaloviral infection; other inflammatory diseases accompanied by enhanced endogenous interferons; autoimmune diseases; and malignant diseases (hematologic malignancies, tumors of the genitourinary tract, and pediatric cancer) (Muller

*et al.*, 1991). The use of neopterin in non-HIV diseases goes on increasing (Murr *et al.*, 1999; Fahey, 1998; Nishanian *et al.*, 1998). Nevertheless because neopterin is increased in other infections include tuberculosis, neopterin is useful to be the prognostic marker for HIV and tuberculosis coinfection (Fahey, 1998).

Neopterin and other immune activation soluble markers such as beta-2-microglobulin or tumor necrosis factors have advantage for stability on storage of serum or plasma specimens. Moreover, it can be measured in non invasive specimens as urine and oral fluids (Murr *et al.*, 1999; Nishanian *et al.*, 1998). These specimens can be frozen for either batch analysis or repeatable analysis.

In conclusion, the detection of immune activation markers, for example, neopterin in serum, urine or oral fluid, are valuable to be disease indicators. The value of soluble markers in assessing the course of HIV disease is noted, especially in situations where expensive technology for CD4 cell and viral load measurements are not available. Neopterin is valuable for other inflammatory disorders as well.

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