Table 2
Clinical characteristics of six patients with HIV/TB co-infection.

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age in years/sex	42/Male	47/Male	37/Male	46/Female	30/Male	44/Male
CD4+ T cell count at TB diagnosis, cells/μl	46	198	19	321	94	344
CXR findings at TB diagnosis	Non-cavitary	Non-cavitary, infiltrates, pleural effusion	Non-cavitary, pleural effusion	Non-cavitary	Non-cavitary	Cavitary
Site of TB	PTB	PTB + EPTB (meningeal)	PTB	PTB + EPTB (colitis)	PTB + EPTB (lymphatic)	PTB
Treatment regimen for TB	2HRZE/4HR	2HRZE/4HR	2HEOS/18HE	2HRZE/4HR	2HRZE/4HR	2HRZE/4HR
HAART initiation during study period[a] (regimen)	Yes (d4T,3TC,NVP)	No	Yes (d4T,3TC,EFV)	No	No	No
Outcomes after 6-9 mo of anti-TB treatment	Cure	Cure	On treatment	Died[b]	Died[c]	Cure

PTB, pulmonary tuberculosis; EPTB, extrapulmonary tuberculosis; 2HRZE/4HR, treatment regimen consisted of the 2-months (mo) initial phase of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) followed by the 4-months continuation phase consisted of isoniazid and rifampicin; 2HEOS/18HE, treatment regimen consisted of the 2-months initial phase of isoniazid, ethambutol, ofloxacin (O) and streptomycin (S) followed by the 18-months continuation phase consisted of isoniazid and ethambutol; d4T, Stavudine; 3TC, Lamivudine; NVP, Nevirapine; EFV, Efavirenz; HAART, highly active antiretroviral therapy.
[a]HAART initiated 2 months after starting anti-TB treatment.
[b]After 87 days of anti-TB treatment.
[c]After 4 days of anti-TB treatment.

HIV+HAART+ (p=0.07). Among patients with HIV+HAART+, the median time interval between initiation of HAART and enrollment was 35 months (range 14-56 months). The baseline and follow-up characteristics of the 6 patients with HIV+TB+HAART- are shown in Table 2. Of these 6 patients, 3 had pulmonary TB and 3 had both pulmonary and extra-pulmonary TB, 2 of them died during anti-TB treatment with a principal diagnosis of disseminated TB. Among the remain patients, 3 were considered to be cured and 1 patient was still undergoing TB treatment after 6-9 months based on National Tuberculosis Program (NTP) guidelines. Of the 3 patients that could be followed-up, 1 patient with a baseline $CD4^+$ cell count<200 cells/μl had started HAART 2 months after anti-TB treatment. Twelve patients with TB and 3 patients with HIV+TB+HAART- were able to be followed-up after 6-9 months of anti-TB treatment and were considered as cured according to the standard criteria.

This study was approved by the Ethical Review Committee for Research on Human Subjects, Ministry of Public Health, Thailand (Reference number 15/2550) and the National Center for

Global Health and Medicine, Japan (Reference number 415). Written informed consent was obtained from all subjects prior to enrollment.

Blood samples

Blood samples were collected in ethylene diaminetetraacetic acid (EDTA) vacutainer tubes from patients and healthy controls at the time of enrollment and after 6-9 months of anti-TB treatment when they were considered as cured. After centrifugation at 1,000*g* for 10 minutes at room temperature, the plasmas were collected and kept at -80°C until used.

Determination of full-length and N-half OPN by ELISA

The levels for full-length (F-OPN) and N-terminal fragment OPN (N-half OPN) were determined with a sandwich ELISA kit according to the manufacturer's instructions (IBL, Gunma, Japan). The tests were done in duplicate and the concentrations of F-OPN/N-half OPN were calculated from a linear equation for each standard curve developed with recombinant human F-OPN/N-half OPN. The subtracted absorbance below zero was considered as zero. The lower detection limits of the F-OPN and N-half OPN assay kits were 3.3 ng/ml and 92.7 pg/ml, respectively.

Determination of cytokines, a chemokine and CRP

IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40), IL-10 and IL-15 levels in plasma were determined using sandwich ELISA kits according to the manufacturer's instructions. The tests were done in duplicate and the concentrations of cytokines/chemokines were calculated from a linear equation for each standard curve. The subtracted absorbance below zero was considered as zero. The lower detection limits of the assays were 4.7 pg/ml for IFN-γ (BD Biosciences Pharmingen, San Diego, CA), 7.8 pg/ml for IP-10 (BD Biosciences Pharmingen), 12.5 pg/ml for IL-18 (MBL, Nagoya, Japan), 62.5 pg/ml for IL-12/IL-23 (p40) (BioLegend, San Diego, CA), 3.9 pg/ml for IL-10 (BioLegend) and 4.0 pg/ml for IL-15 (BioLegend).

Highly sensitive C-reactive protein (CRP) levels in plasma were measured by means of particle enhanced immunonephelometry using the BN system (*Cardio*Phase® *hs*CRP, Dade Behring, Newark, DE). The lower detection limit was 148 ng/ml. Values below this level were considered equal to 148 ng/ml. A level of 3,000 ng/ml in the serum was considered as the upper limit of normal.

Statistical analysis

Statistical analysis was performed using SPSS software version 17.0. The data were expressed as medians and ranges. Since not all the parameters exhibited normal distribution, comparison between two independent groups was performed using the nonparametric Mann-Whitney *U* test, and comparison between the two dependent groups was performed using the nonparametric Wilcoxon signed-ranks test. The correlations among the F-OPN, N-half OPN and T cell response-associated molecules were analyzed using a Spearman's rank correlation test. A p-value <0.05 was considered significant.

RESULTS

Circulating F-OPN levels in TB

The plasma F-OPN levels from patients with TB (251.9-959.9 ng/ml) and HIV+TB+HAART-(853.2-4,005.4 ng/ml) were significantly higher than in patients with HIV+HAART- (209.5-450.8 ng/ml) ($p<0.01$, $p<0.01$, respectively), HIV+HAART+ (141.2-655.1 ng/ml) ($p<0.01$, $p<0.001$, respectively) and HC

(37.3-517.8 ng/ml) ($p<0.000001$, $p<0.001$, respectively) (Fig 1a). The plasma F-OPN levels in patients with HIV+TB+HAART- were significantly higher than in patients with TB ($p<0.001$). Although the N-half OPN levels were below the detection sensitivity (92.7 pg/ml) in many study subjects (Fig 1b), the N-half OPN levels in patients with TB tended to be higher than in patients with HIV+HAART- and HIV+HAART+ and HC. Half of patients with HIV+TB+HAART- had even higher N-half OPN levels than patients with TB ($p<0.01$).

Changes in circulating IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40), CRP and IL-10 in TB

Before anti-TB treatment, the plasma levels of IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40) and CRP in patients with TB were significantly higher than in HC ($p<0.0000001$, $p<0.01$, $p<0.0001$, $p<0.00001$, and $p<0.0000001$, respectively), whereas IL-10 levels in patients with TB were significantly lower than in HC ($p<0.01$) (Fig 1c-1h). Patients with TB had significantly higher plasma IFN-γ, IP-10, IL-18 and CRP levels than patients with HIV+HAART+ ($p<0.001$, $p<0.0001$, $p<0.01$ and $p<0.000001$, respectively), and they had significantly higher IFN-γ and CRP levels than patients with HIV+HAART- ($p<0.01$ and $p<0.0001$, respectively). Patients with TB had significantly lower IL-12/IL-23 (p40) levels than patients with HIV+HAART- ($p<0.001$). Similarly, the plasma IFN-γ, IP-10, IL-18, IL-10 and CRP levels in patients with HIV+TB+HAART- were significantly higher than in HC ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.01$ and $p<0.001$, respectively), patients with HIV+HAART- ($p<0.01$, $p<0.01$, $p<0.01$, $p<0.01$ and $p<0.01$, respectively) and patients with HIV+HAART+ ($p<0.01$, $p<0.001$, $p<0.01$, $p<0.01$ and $p<0.01$, respectively). The plasma IP-10, IL-18, IL-12/IL-23 (p40) and IL-10, but not IFN-γ and CRP levels in patients with HIV+TB+HAART- were significantly higher than in patients with TB ($p<0.001$, $p<0.01$, $p<0.05$ and $p<0.01$, respectively). The circulating levels of IL-15 were below the detection sensitivity of 4.0 pg/ml in almost all studied subjects, causing no significant differences (data not shown).

Correlations among circulating F-OPN, N-half OPN, IFN-γ, IP-10, IL-18, CRP and clinical parameters in tuberculosis cases

Correlations among plasma F-OPN, N-half OPN, IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40), IL-10, IL-15 and CRP levels before anti-TB treatment were analyzed in patients with TB. Plasma F-OPN correlated significantly with N-half OPN ($r=0.508$, $p<0.05$), IP-10 ($r=0.500$, $p<0.05$) and IL-18 ($r=0.568$, $p<0.01$); whereas plasma F-OPN did not correlate with IFN-γ, IL-12/IL-23 (p40), IL-10, IL-15 or CRP. Positive correlations were also found between plasma levels of IP-10 and IFN-γ ($r=0.525$, $p<0.05$), IP-10 and IL-18 ($r=0.527$, $p<0.05$) and IL-18 and CRP ($r=0.519$, $p<0.05$). In patients with HIV+TB+HAART-, plasma F-OPN levels correlated significantly with IP-10 and IL-18 levels ($r=0.943$, $p<0.01$ and $r=0.829$, $p<0.05$, respectively).

The correlations between T cell response-associated molecules and the number of WBCs, lymphocytes, monocytes, $CD4^+$ T cells, $CD8^+$ T cells and $CD4^+/CD8^+$ ratio were analyzed in patients with TB. There were significant positive correlations between plasma F-OPN levels and WBC counts ($r=0.508$, $p<0.05$), CRP and WBC counts ($r=0.651$, $p<0.01$) and negative correlations between IFN-γ and $CD4^+/CD8^+$ ratios ($r=-0.474$, $p<0.05$), IP-10 and $CD4^+/CD8^+$ ratios ($r=-0.69$, $p<0.001$).

Circulating OPN, IFN-γ, IP-10 and CRP levels after anti-TB treatment

Plasma F-OPN, IFN-γ, IP-10, IL-18,

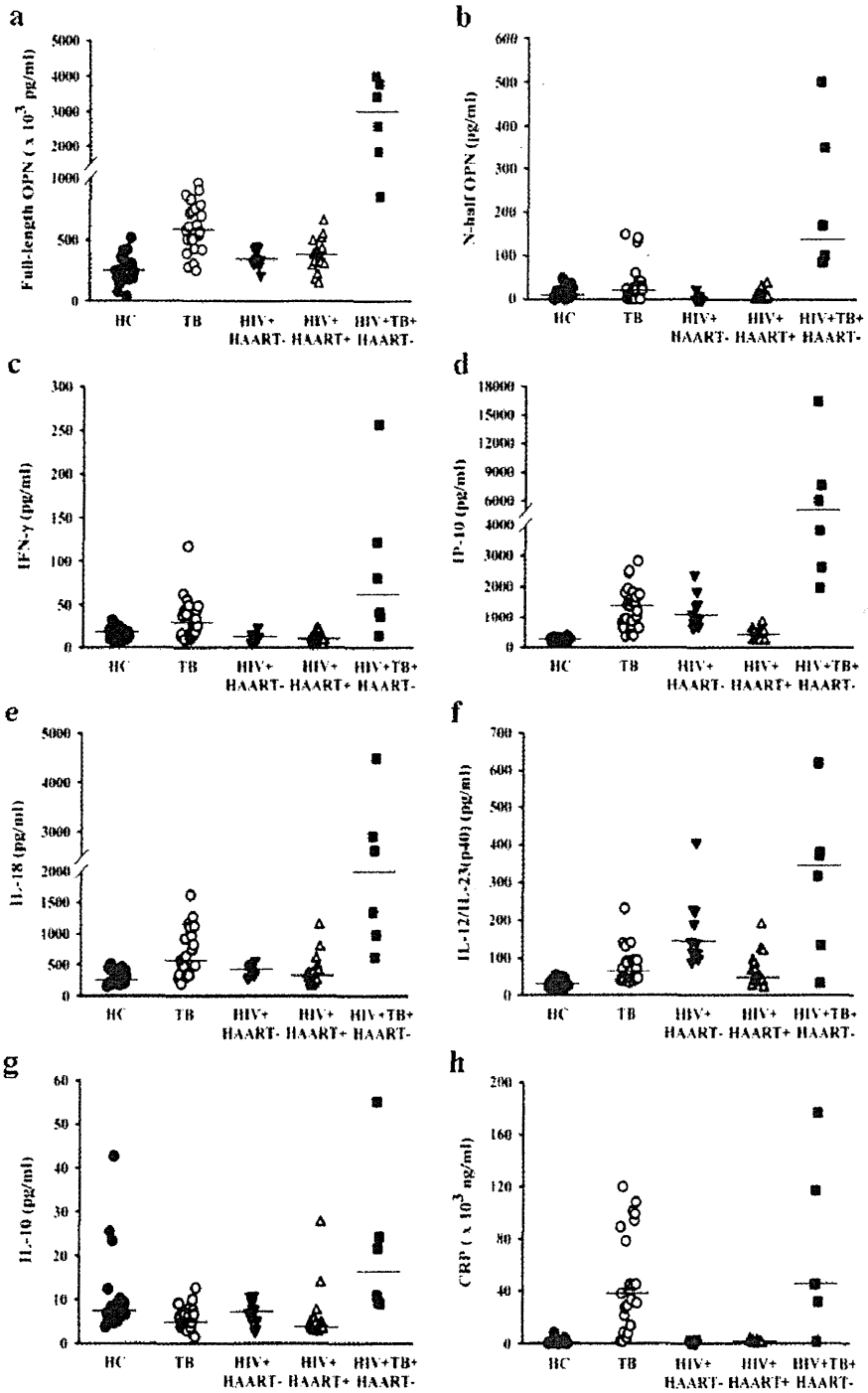

Fig 1–Circulating full-length OPN (a), N-half OPN (b), IFN-γ (c), IP-10 (d), IL-18 (e), IL-12/IL-23 (p40) (f), IL-10 (g) and CRP (h) levels in patients with tuberculosis (TB) and HIV/TB co-infection without HAART (HIV+TB+HAART-). HIV patients without HAART (HIV+HAART-) and with HAART (HIV+HAART+) were tested in comparison. Healthy individuals (HC) were used as controls. Bars represent the median values. The horizontal lines represent the lower limits of each measurement.

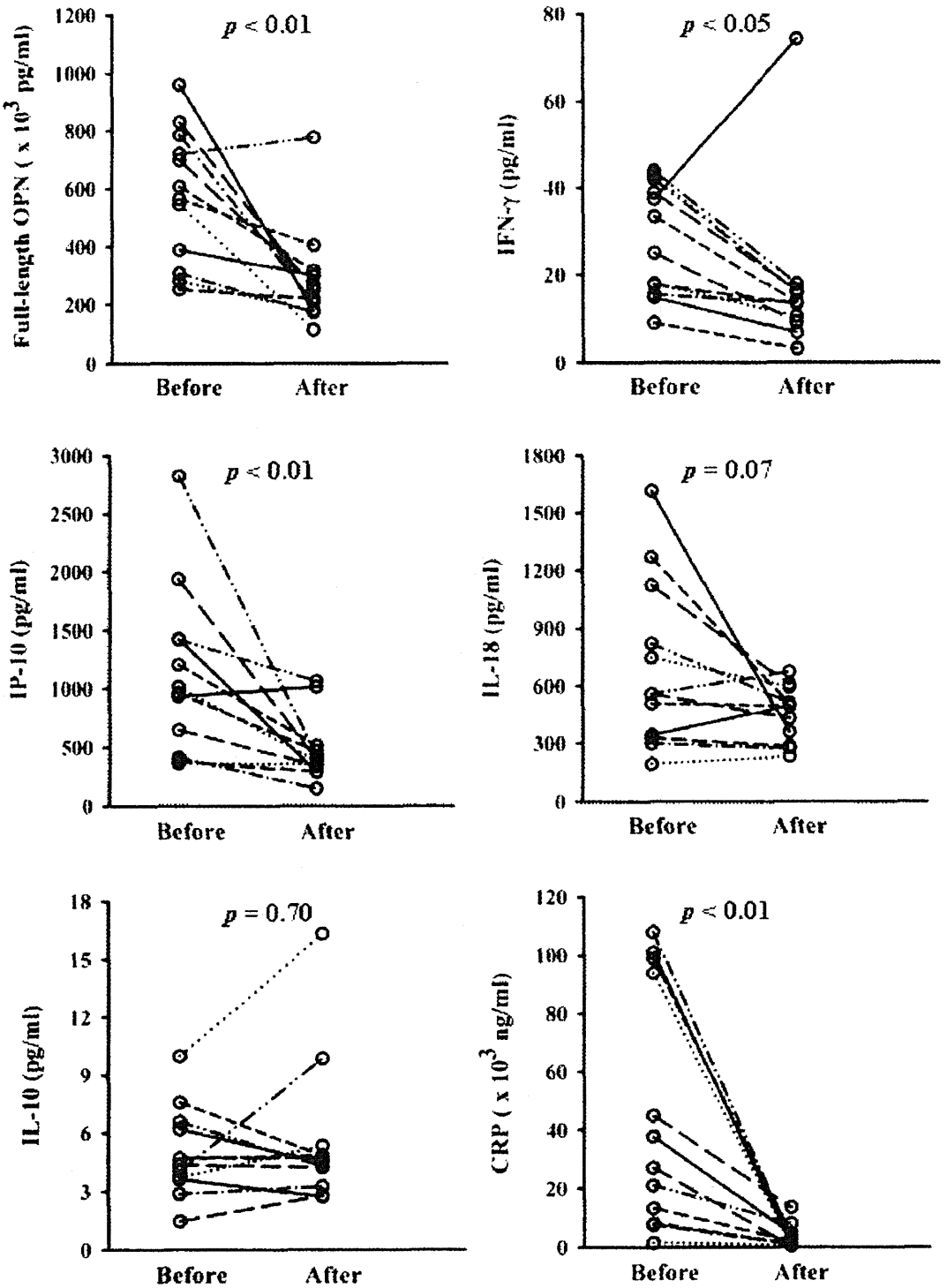

Fig 2–Circulating full-length OPN, IFN-γ, IP-10, IL-18, IL-10 and CRP levels among patients with active pulmonary TB before and after anti-TB treatment.

IL-12/IL-23 (p40), IL-10, IL-15 and CRP levels before and after 6-9 months of anti-TB treatment in the 12 patients with TB and in the 3 patients with HIV+TB+HAART- were evaluated. Significant decreases in plasma F-OPN, IFN-γ, IP-10 and CRP levels were seen in patients with TB after treatment ($p<0.01$, $p<0.05$, $p<0.01$ and $p<0.01$, respectively) (Fig 2). Although plasma IL-18 levels decreased in some TB patients after treatment, the change was not significant.

Plasma F-OPN, IFN-γ and CRP levels in patients with HIV+TB+HAART- tended to decrease after anti-TB treatment. After treatment, clinical improvement, negative sputum microscopy examinations and normal chest radiographs were observed.

DISCUSSION

To address the role of OPN in patients with TB, circulating F-OPN, N-half OPN and other cytokines and chemokine levels were evaluated along with clinical parameters in Thai patients with active pulmonary TB and HIV/TB co-infection. Circulating F-OPN, IFN-γ and CRP levels were significantly elevated in patients with active pulmonary TB and the levels decreased after effective anti-TB treatment. High concentrations of F-OPN, N-half OPN, IFN-γ, IP-10, IL-18 and IL-10 found in the plasma of patients with HIV/TB co-infection were unexpected, although this was a small-scale study. Levels of N-half OPN were much lower than those of F-OPN in all groups. Plasma levels of F-OPN correlated well with IP-10, IL-18 and N-half OPN levels among patients with active TB.

The high F-OPN levels in TB patients suggested a role for circulating F-OPN in disease activity among TB patients. Elevated circulating F-OPN levels in pulmonary TB patients is consistent with previous studies (Koguchi *et al*, 2003; Inomata *et al*, 2005). This may be partly due to leakage from granuloma sites evidenced by accumulation of OPN proteins in lung tissue sections from TB patients (Nau *et al*, 1997) and by abundant OPN expression in lymph nodes with well-formed granulomas (Nau *et al*, 2000). However, elevated circulating F-OPN and N-half OPN in patients with HIV/TB co-infection was not expected. HIV/TB co-infection is known to be associated with failure of granuloma formation and failure to control *M. tuberculosis* infection, thereby leading to mycobacterial dissemination (Corbett *et al*, 2003). The contribution of HIV infection to elevated circulating F-OPN is known and these levels correlate with HIV-induced CNS dysfunction, particularly in HIV-associated dementia, a severe neurocognitive abnormality that commonly occurs during the late stages of HIV infection (Burdo *et al*, 2008). Without receiving HAART, HIV infection chronically activates the host immune system to maintain a defense that only partially controls infection (Fauci, 1996), but chronic activation and replication, as well as storage of virus, leads to pathological consequences that may stimulate the production of various mediators of immune activation, including OPN. Collectively, prominent levels of circulating F-OPN in HIV/TB co-infection may not indicate disease status of effective granuloma formation but rather reflect spread of active TB lesions, large numbers of pathogens in the body or synergistic immune activation due to HIV/TB co-infection. F-OPN levels may not be equivalent to TB-associated inflammation simply measured by CRP because F-OPN levels did not correlate with CRP levels in the TB group.

The introduction of HAART among

HIV-infected patients usually results in the gradual reconstitution of the immune system (Weiss *et al*, 1999). HAART induced changes in the expression of many pro-inflammatory cytokines, including OPN in lymph nodes of HIV infected individuals 1 month after initiation (Li *et al*, 2004) but persistently elevated levels of circulating F-OPN during 6 months of HAART were observed (Chagan-Yasutan *et al*, 2009). In line with the latter findings, in this study, no differences in circulating F-OPN levels between HIV patients with or without HAART were found, despite a possible alteration in immune status with HAART. Different results are possibly due to differences in disease stage, regimen and duration of HAART.

Levels of circulating N-half OPN were much lower than those of F-OPN among all groups, and may not be helpful for monitoring disease activity. N-half OPN is generally more potent in causing cell migration and adhesions at the site of disease than in the uncleaved full-length form (Senger *et al*, 1994). In the synovial fluid of patients with rheumatoid arthritis (RA), N-half OPN has been detected at lower levels than F-OPN (Hasegawa *et al*, 2009). This indicates that N-half OPN exists at lower levels than its full form even at the site of inflammation. N-half OPN was detected in urine but not plasma from patients with RA at much lower levels than F-OPN (Shio *et al*, 2010). N-half OPN may not be stable in body fluids, including plasma, or is barely produced in tissues through strict regulation of thrombin/anti-thrombin balance. Thus, investigation regarding the functional form of OPN in TB and HIV/TB co-infection is further necessary when a more sensitive assay system is developed.

Elevation of circulating F-OPN, IFN-γ, IP-10 and IL-18 levels was documented in patients with active pulmonary TB. The results of circulating F-OPN, IFN-γ and IL-18 levels in patients with TB are consistent with other studies (Verbon *et al*, 1999; Morosini *et al*, 2003; Inomata *et al*, 2005). The finding of lower circulating IL-10 levels among TB patients than healthy controls is in contrast to some other studies (Verbon *et al*, 1999; Morosini *et al*, 2003; Deveci *et al*, 2005). This variability may result from a different status of healthy controls, in that all were negative on the interferon-gamma release assay (IGRA) in our study, whereas other studies consisted of controls with both positive and negative tuberculin skin tests (TST) (Morosini *et al*, 2003; Inomata *et al*, 2005). IL-10 levels in healthy controls in this study may have been affected by simultaneous infection with helminthes or tropical diseases, as is often seen in developing countries (Borkow and Bentwich, 2004). TB patients have different clinical characteristics, but only pulmonary TB patients with sputum smears positive for acid-fast bacilli (AFB) were recruited into this study, whereas another study included patients with both pulmonary and extra-pulmonary TB (Verbon *et al*, 1999).

The present results showed elevated IFN-γ and IP-10 levles were found in TB patients similar to previous studies (Juffermans *et al*, 1999; Azzurri *et al*, 2005; Djoba Siawaya *et al*, 2009). The present study demonstrated, for the first time, positive correlations between levels of F-OPN and IP-10, between IP-10 and IL-18 and between IP-10 and IFN-γ in patient with TB. Our findings of no correlations between circulating F-OPN and IFN-γ, between F-OPN and IL-12 and between IFN-γ and IL-12 are in contrast with some previous studies (Inomata *et al*, 2005; Pokkali and Das, 2009). Further studies are needed. OPN was found to be elevated

along other Th1-related molecules in patients with active TB.

In patients with TB, a significant decrease in circulating F-OPN, IFN-γ, IP-10, CRP levels and a trend toward a decrease in IL-18 levels were observed 6 to 9 months after anti-TB treatment. Furthermore, a decrease in circulating F-OPN, IFN-γ and CRP in 3 HIV/TB co-infected patients after completing treatment suggests these molecules may be useful for evaluating TB disease activity and monitoring response to treatment, as has been shown in previous studies (Koguchi *et al*, 2003; Inomata *et al*, 2005). However, discrepancies may occur (Verbon *et al*, 1999; Inomata *et al*, 2005; Djoba Siawaya *et al*, 2009) and caution is needed to interpret the results.

In conclusion, the present study confirmed the possible contribution of OPN for evaluating pulmonary TB disease activity, particularly in HIV/TB co-infected patients in association with Th1 response-related molecules. Clinically, the elevated OPN, IFN-γ and CRP levels and their decline after successful anti-TB treatment suggests circulating levels of F-OPN and Th1 response-related molecules, including IFN-γ, may be useful to determine expansion of active TB lesions and/or pathogens and may serve as markers of disease activity before and during treatment.

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Predictors of antiretroviral therapy regimen changes in Northern Thailand

Abstract:

Background: Chiang Rai province, Northern Thailand is a highly HIV prevalent area where nevirapine-based regimen is first line therapy. This study aimed to determine the rate, predictors, and reasons for highly active antiretroviral therapy (HAART) regimen change.

Methods: A total of 4981 HIV patients who attended 17 HIV clinics of Chiang Rai province were enrolled from 2006 to 2008. In the first year of follow up, we observed the HAART regimen changes rate and predictors of changes were investigated using Cox-proportional hazard model.

Result: Overall HAART regimen change rate within the first year was15.4% (95% Confidence Interval 14.4-16.4%) of 4981 The regimen change rate was higher in female than in male (17.6%vs.12.9%). Patients on efavirenz-based regimen underwent significantly higher rate of regimen change (20.3 %) than nevirapine-based (14.9%) and protease inhibitors-based regimen (11.9%). The regimen change rate became higher in 2007 and 2008 than in 2006 and higher at the provincial hospital than district community hospitals. Out of the 765 patients who underwent regimen change, the most common cause was adverse effect (83.5%; n=639)

Discussion; Strategy should be developed by the prospective trial to minimize the side effect, and reducing the regimen changes in order to improve the treatment outcomes.

Introduction:

HAART (Highly Active Antiretroviral Therapy) could prolong the life expectancy and quality of life of HIV-infected patients globally [1]. Sustainability of immunological and virological response achieved by a first-line regimen is important [2]. However, drug resistance, short-term and long-term side effects of antiretroviral drugs may lead to changes in first- line drugs regimen or switch to second-line regimens [3]. The recommendations on the timing of HAART regimen change differ by guidelines. The World Health Organization (WHO) HIV treatment guideline published in 2010 recommends reservation of second-line drugs unless there is strong reason to change the first-line regimen, while the European and British guidelines and Department of health and human service, U.S.A. (DHHS) guidelines favor more sensitive switch and change of HAART to prevent the accumulation of thymine analogue mutation (TAM) [2, 4-6].

Currently, ART (antiretroviral therapy) coverage in Thailand has extended to more than two thirds of those in need. Thailand has developed its own national HIV treatment guideline updated in 2007, 2008 and 2010 [7-9]. Until 2008, fixed combination of d4T/3TC/NVP (GPO-VIR S 30), fixed combination of AZT/3TC/NVP (GPO-VIR Z 250), and d4T+3TC+EFV were first line recommended HAART regimen by Thailand national treatment guideline [7-8].

Observational studies [10-12] have reported different regimen change rate at HIV cohort of different regions but mostly in developed countries using protease inhibitor (PI) based regimen to avoid the side effects of Non-Nucleic acid Reverse Transcriptase Inhibitor (NNRTI), in particular nevirapine (NVP). However, developing countries still use NVP due to its lower cost. So, it is important to know the nature of regimen changes, their cause of changes in a setting of limited resource, most of the patients were treated by NVP-based regimen.

We aimed to investigate the rate and predictors of ART regimen changes within first year of HAART initiation in newly treated HIV patients, and to compare the impact of regimen changes on treatment outcome between patients who underwent regimen changes within one year and those who did not.

Materials and methods

Study design and data collection

A retrospective cohort was constructed enrolling HIV patients treated in HIV clinics of 17 hospitals in Chiang Rai province between 2006 and 2008. We used part of the provincial AIDS program database at Chiang Rai Provincial Health Office with the supplemental information and validation of data with the hospital medical records. Inclusion criteria to comprise the study cohort were HIV patients receiving HAART of first line regimens, and treated for the first time. Those aged less than 15 years, lost to follow up or died before one year follow up were excluded from the study. Those who were receiving second line therapy at the start of NAP (National AIDS Program) were also excluded.

Total follow-up period was two years. Within the first year of follow-up, study cohort was observed for event of regimen changes. Based on whether a patient's HAART regimen has been changed within the first year of observation, the cohort was divided into RCG (regimen change group) and NCG (non regimen change group) groups. These two groups were followed into the second year to compare the treatment outcome by immunological status and virological response.

Definitions of HAART regimen change:

Regimen change was defined as change in at least one drug within one year of treatment. Dose modifications were not considered as change. .

Laboratory monitoring tools;

HIV RNA viral load (VL) was checked once a year and CD4 counts were checked every six months for patients who were provided HAART through the National Health Security Office (NHSO). Under this scheme, VL testing was provided only after one year of treatment initiation and thus baseline VL was not available.

CD4 count: CD4 count was checked by the dual-platform flow cytometry at the provincial hospital and one major district hospital.

HIV RNA Viral load: HIV RNA viral load test were done by real-time PCR. The minimum detection limit was 40 copies /ml. Undetectable viral load was considered as HIV RNA viral load less than 50 copies ml according to current guidelines [2, 4].

Statistical analysis: The predictors of ART regimen changes were analyzed using Cox-proportional hazard model to determine the crude and adjusted hazard ratio (AHR) and its 95% confidence interval (CI). Variable which have statistical significance of P-value less than 0.2 in univariate analysis were included in the multivariate models. Mann Whitney's test was used to analyze the baseline quantitative variables. All tests were two tailed. Statistical significance was considered as P value less than 0.05. STATA release 10 was used for data analysis.

Ethical consideration

Ethical approval to conduct this study was obtained from the Chiang Rai Prachanukroh Hospital ethical committee, Ministry of Public Health, Thailand

Results

The study cohort comprised 4891 patients. Median age was 36 years (IQR: interquantile range 31-41) and 42% of the patients were female. All patients were Thai citizens. 96.6% of patients were supported by the health security system including NHSO (89.0%),

social security for employee/employer (4.4%), and government officer (0.4%). HAART was started in HIV patients with CD4 count less than 200 cells/μl according to national HIV treatment guidelines at that time [7, 9].

765 patients (15.4%, 95%CI 14.4-16.4%) of 4891 underwent ART changes within one year of first time HAART initiation in health service system. (Table 1) Regimen change rate was significantly higher in female patients compared to male patients, and those treated in provincial hospital compared to district hospitals. In addition, a secular trend was observed where regimen change was significantly more frequent in 2008 compared to 2006. Patients receiving EFV-based regimen had significantly higher change rate (20.3%, 95/469) than those on NVP-based regimen (14.9%, 662/4445). Patients receiving d4T+3TC backbone underwent higher rate of regimen change compared to AZT+3TC.

With multivariate analysis by Cox-proportional hazard model, female sex (AHR 1.48), EFV based regimen (AHR 1.48), d4T+3TC backbone (AHR 1.44), treatment at provincial hospital (AHR 1.41), ART started in 2008 (AHR 2.28) were significant predictors of the ART regimen change within first year of HAART initiation. Cumulative Percentage of HAART regimen change by initial type based on third agent were shown in figure 1.

Out of the 765 patients who underwent regimen change, the most common cause reported was adverse effect (83.5%; n=639) followed by treatment failure (6.0%; n=46), clinician`s decision to prevent side effect (3.9%; n=30), drug interaction (3.1%; n=24), hepatitis B co- infection (2.1%; n=16), and pregnancy (1.3%; n=10) (Table 3).

Based on presence of regimen change in the first year after treatment initiation, the cohort was divided in to RCG (regimen changed group) and NCG (non regimen changed group). These two groups were followed into the second year and immunological and virological outcome were compared. Overall 87.8% of the entire cohort was having CD4 higher than 200cells/ul at two years after initiation of HAART. The proportions patients in different CD4 count categories were not different statistically, i.e., 12.1% in RCG vs. 12.3% in NCG had CD4 count under 200 cells/ul (P=0.89). Overall, 87.8% of cohort achieved undetectable viral load at one year after regimen change. 84.7% of RCG and 89.2% of NCG had undetectable viral load in follow-up after changes.

Discussion

The regimen change rate within first year of HAART initiation in Chiang Rai was 15.4%. The regimen change rate was higher at the provincial hospital compared to district hospitals. Difference in laboratory facility for identification of drug resistance and immunological failure and difference in level of care by specialist physicians may have contributed to the difference observed. Female patients were more prone to regimen change compared to male.

HAART regimen change rate in first years were reported by different cohorts in the existing literature; 37% in Swiss cohort from 2000-2005[10], 18.4% in USA [11], 36.1% in Italian cohort from 1997-2007[13], and 28% throughout the Caribbean and Latin America from 1996-2007 [14]. Treat Asia cohort reported 29% regimen change rate among 1846 patients on median follow up for 3.2 years [15]. Compared to these figures, HAART regimen change rate in our study was relatively low.

Reported reasons for changes are variably contributed by adverse effect and treatment failure among cohorts of developed and developing countries [11, 13]. Adverse effect proportion is larger in developing setting and failure proportion is larger in developed probably because of earlier detection [10-11, 13].

Chiang Rai is a resource-limited area with high burden of HIV infection. D4T/3TC/NVP regimen which is the first-line regimen in this setting is well known for side effects. Thailand began a national ARV treatment program in 2000 as the Access to Care program and then expanded this program in 2004 as the National Access to ARVs for People Living with HIV/AIDS (NAPHA). In these Thai national programs in 2000-2007 report 66.9% of the regimen changes were attributable to adverse effects and 21.3% to treatment failure [16]. In our study, the most common reason for regimen change was adverse effects and few were attributable to treatment failure.

The majority of the patients in our study were provided HAART under the NHSO which aims for universal coverage. This scheme only allows viral load testing at six months after HAART initiation and yearly thereafter. Good adherence to ART of patients which is strongly reinforced by network of PLWH in Chiang Rai [17] might also contibuted. These factors could have lead to the relatively low rate of regimen change observed

Our study result showed that EFV-based regimen is more likely to undergo regimen change within first year (AOR= 1.63). Although many previous study results are more favorable for EFV compared to NVP in term of efficacy and safety profile [18-19], similar finding of higher regimen change rate with EFV based regimen was reported by a secondary analysis of a cohort study in South Africa [20]. Treat Asia study [15] also reported 3 or more ART with NNRTI, no PI (other than d4T/3TC/NVP), which EFV-based regimen is 86.7% (686/791), has higher regimen change rate compared with d4T/3TC/NVP as multivariate rate ratio (RR)= 1.64 (95%CI=1.38-1.96).

The d4T+3TC back bone was also a significant predictor for regimen change. Adverse effects of d4T are reported and d4T is expected to be withdrawn from the Thai national program in the coming two years [8].

Swiss cohort study [10] reported difference of regimen change in patients with CD4+ cell counts more than 350 /mm^3 compared with 200-350, it was not different between <200 and 200-350. The other studies [11, 13, 14,] did not have association of CD4+ T cell counts level and regimen change even 350 cells/mm^3 cut-off. Thai guideline recommended to start HAART in asymptomatic HIV-infected patients at CD4+ T cell counts <200 cells/mm^3 in 2008 recommendation [8] which has increased <350 cells/mm^3 in 2010 [9], thus we do not have association with CD4 + T cell counts level is consistent.

In Thailand there are several pharmacogenetic study to investigate the biomarker for side effect of Nevirapine [21] and Stavudine (d4T) [22]. This information should be used for the prospective trial to minimize the side effect, reducing the regimen changes, and improve the treatment outcomes [23].

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Table 1: HAART regimen change rate within first year of initiation by baseline patient characteristic

Characteristic N=4981	Regimen change rate (%)	Change /total number	P value[1]
Overall	15.4%	765/4981	
Age (years)			
>50	19.4%	49/253	0.07
15-50	15.1%	716/4728	
Base line CD4 (cells/ul)			
>50	15.6%	665/4260	0.54
0-50	16.7%	70/418	
Missing	9.9%	30/303	
Gender			
Male	12.9%	308/2389	<0.001
Female	17.6%	457/2592	
HAART regimen[2]			
NVP- based	14.9%	662/4445	<0.01
EFV-based	20.3%	95/469	
PI-based	11.9%	8/67	
NRTI Backbone			
Stavudine (d4T)+3TC	15.9%	652/4095	0.02
AZT+3TC	12.8%	113/886	
Presence of OI[3] at HAART initiation			
Yes	16.3%	17/104	0.82
No	15.5%	748/4820	
Missing	0.0%	0/57	
Type of hospital			
Provincial	19.1%	174/913	<0.01
District	14.5%	591/4068	
Year of HAART initiation			
2006	11.0%	24/218	<0.01
2007	14.7%	539/3659	
2008	18.3%	202/1104	

[1]P-value : calculated by chi-square test. [2]Types of HAART regimen based on the third agent. [3]OI: WHO stage 3 and 4 opportunistic infection

Table 2: Cox proportional hazards model analyses for predictors of HAART regimen changes within one year of treatment initiation.

Characteristic N=4981	Unadjusted Hazards ratio (95%CI[1])	Adjusted[2] Hazards ratio (95%CI)	P
Age (years)			
>50	1	1	
15-50	1.35 (1.01-1.8)	1.28 (0.96-1.71)	0.10
Base line CD4 (cells/ul)			
>50	1		
0-50	1.2 (0.94-1.54)		
Gender			
Male	1	1	
Female	1.4 (1.22-1.62)	1.48 (1.28-1.72)	<0.001
HAART regimen[3]			
NVP-based	1	1	
EFV-based	1.4 (1.13-1.74)	1.48 (1.19-1.84)	<0.001
PI-based	0.79 (0.4-1.59)	0.77 (0.38-1.55)	0.46
NRTI Backbone			
AZT+3TC	1	1	
D4T+3TC	1.29 (1.06-1.58)	1.44 (1.17-1.77)	<0.01
Presence of OI[4] at HAART initiation			
Yes	1		
No	1.18 (0.73-1.91)		
Type of hospital			
Provincial	1	1	
District	0.74 (0.63-0.88)	0.71 (0.6-0.84)	<0.001
Year of HAART initiation			
2006	1	1	
2007	1.44 (0.96-2.17)	1.72 (1.14-2.6)	0.01
2008	1.96 (1.28-2.99)	2.28 (1.49-3.49)	<0.001

[1]CI: Confidence Interval [2]Adjusted for all other covariates in the model. [3]Types of HAART regimen based on third main agent. [4]OI: Who stage 3 and 4 opportunistic infection.

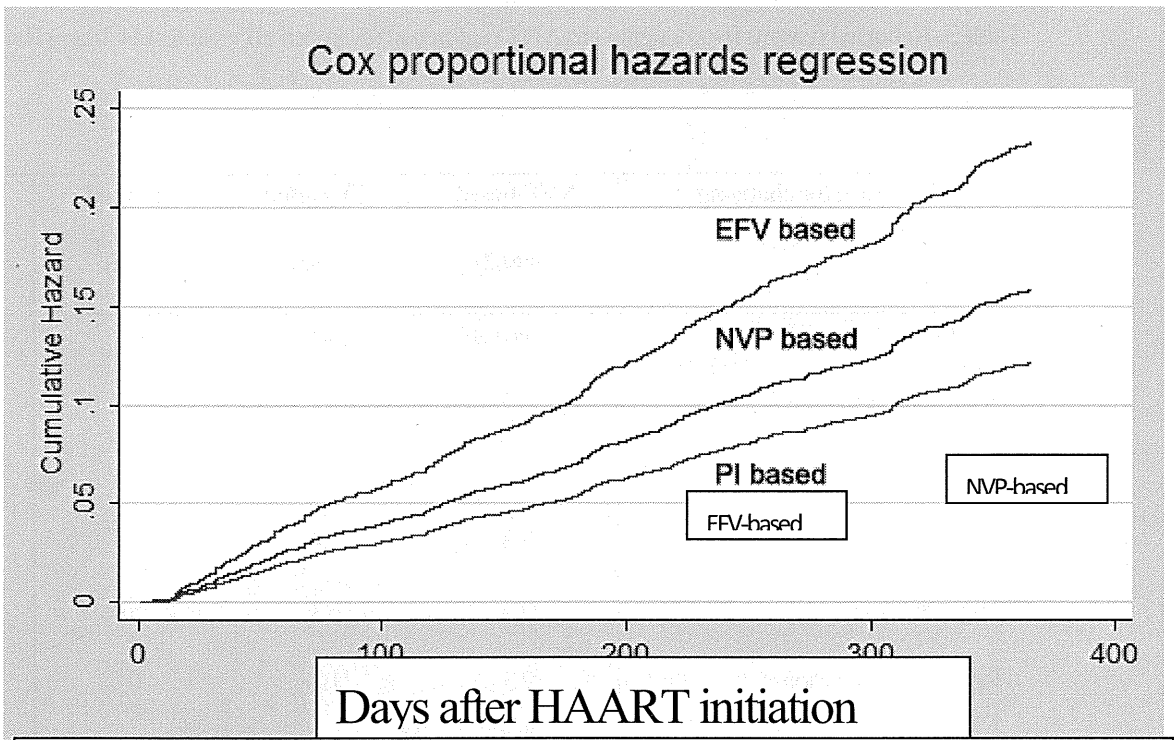

Figure 1. Cumulative Percentage of HAART regimen change by initial type based on third agent

Table 3: Reported reasons for changing HAART regimen at seventeen HIV clinics of Chiang Rai Province

Reported reason for changing regimen	NVP-based (n=662)	EFV-based (n=95)	PI-based (n=8)	Total (N=765)
Adverse effect	84.3	80.0	62.5	83.5
Treatment failure	6.5	3.2	0	6.0
Clinicians' decision to prevent side effect	3.3	7.4	12.5	3.9
Drug interaction	2.4	6.3	25.0	3.1
Hepatitis B co-infection	2.3	1.1	0	2.1
Pregnancy	1.2	2.1	0	1.3
Total	100.00	100.00	100.00	100.0