Prognostic values of serum IP-10 and IL-17 in patients with pulmonary tuberculosis

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Abstract. *Objective:* To identify patients at high risk of relapse after anti-tuberculosis (TB) therapy or with poor long-term outcomes.

Methods: Fifty-one patients with pulmonary TB: 7 were classified as high association with both cavitations on initial chest radiography and positive sputum smear/cultures after two months of anti-TB treatment (HA group); 19 medium association (MA, one risk alone); and 25 low association (LA, neither risk). Serum interferon (IFN)-γ-inducible protein 10 (IP-10), interleukin-17 (IL-17), and C-reactive protein levels were investigated.

Results: There was a trend towards higher serum IP-10 levels ($p = 0.042$) for HA patients throughout the 6-month treatment period. Month-2 IP-10 levels were higher in the HA than in the MA/LA group (656.2 ± 234.4 vs. 307.6 ± 258.5 pg/ml, adjusted $p = 0.005$). Receiver operating characteristic curves showed that the risk of relapse was well-captured by month-2 IP-10 levels at a cut-off value of 431 pg/ml (AUC = 0.857, 95% CI 0.75–0.97, $p = 0.003$). Month-2 serum IL-17 levels were lower in non-survivors than survivors (15.7 ± 2.9 pg/ml vs. 24.6 ± 8.2 pg/ml, $p = 0.001$). Multivariate analysis demonstrated that a month-2 serum IL-17 level of $\leqslant 17$ pg/ml ($p = 0.026$) was independently associated with all-cause mortality.

Conclusions: Serum IP-10 and IL-17 levels after 2 months of anti-TB treatment may be biomarkers for estimating risk of both cavitation and delayed sputum conversion, and for predicting long-term mortality, respectively.

Keywords: Pulmonary tuberculosis, interferon-γ-inducible protein 10, interleukin-17, caviation, mortality

1. Introduction

Standard anti-tuberculosis (TB) therapy consists of six months of rifampin (RIF) and isoniazid (INH), initially supplemented by two months of pyrazinamide (PZA) and ethambutol (EMB) [1]. The presence of both cavitations and a positive smear/culture for *Mycobacterium tuberculosis* after two months of treatment can amplify the risk of relapse to 20% and above within two years, compared with approximately 5% for patients with only one risk factor and less than 2% for patients with neither risk factor [1–6]. The high relapse rate can be reduced by prolongation of the continuation phase from four to six or seven months [7–10]. The American Thoracic Society has recommended extending 6-month regimens by three months if both risk factors are present [1]. The requirement for biomarkers in assessing treatment response to TB stems from its long and variable natural history, and the non-replicating mycobacterial subpopulations that persist in granulomas and large cavities. Biomarkers that can identify patients at high risk of relapse or long-term outcomes may help in making a clinical judgment on the require-

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ment of prolonged or extensive regimens, and in accelerating drug development by providing validated surrogate endpoints. Some biomarkers have been identified as indicators of bacterial persistence or treatment response, but not yet validated in clinical practice [11, 12].

Interferon (IFN)-γ-inducible protein 10 (IP-10) is a chemokine expressed in both lymphocytes and monocytes after paracrine stimulation of T-cells by IFNs and other proinflammatory cytokines [13,14]. IP-10 is involved in trafficking monocytes and activated T-helper cell type 1 cells to inflamed foci through interaction with the CXC chemokine receptor (CXCR) 3 [15,16]. IP-10 has been identified as an indicator for both infection and curability, but not activity or severity of pulmonary TB in previous studies [17–19]. Interleukin (IL)-17 is produced by a subset of activated CD4+ T cells (Th17), as well as some CD8+ T cells, $\gamma\delta$ T cells, and NKT cells [8–23]. IL-17 may bind to specific receptors (IL-17R) expressed on leukocytes, epithelial cells, vascular endothelial cells, fibroblasts, and mesothelial cells, resulting in neutrophil homeostasis and recruitment through induction of proinflammatory cytokines and chemokine release [26–29]. Up-regulation of IL-17 expression in T lymphocytes has been correlated with active TB [30]. C-reactive protein (CRP) is a non-specific marker of systemic inflammation, and has been linked to the presence of cavitations and treatment response to pulmonary TB [31–33]. The roles of these three serum proteins in estimating risk of cavitation and/or a positive sputum smear/culture or predicting long-term outcomes in pulmonary TB have not yet been determined.

In this study, we investigated whether these serum proteins could serve as biomarkers associated with high risk of cavitation and/or a positive sputum smear/culture after 2-month anti-TB treatment, or linked with poor long-term outcomes, such as death. Therefore, we prospectively followed up these parameters in active pulmonary TB patients for a 6-month treatment period, and correlated them with either the presence of risk factors for relapse (cavitations on chest radiographs (CXR) at diagnosis and a positive sputum smear/culture after two months of anti-TB treatment), or all-cause mortality.

2. Patients and methods

One hundred and twenty-six patients with active pulmonary TB were enrolled prospectively from our out-

patient Pulmonary Clinic at Kaohsiung Chang Gung Memorial Hospital, a medical centre in southern Taiwan, from July 2006 to December 2008. The diagnostic criteria were based on the guidelines of the American Thoracic Society (ATS) [1]. Patients who had true non-tuberculous mycobacterium (NTM) infection (12 patients), poor drug compliance (15 patients), immuno-compromised status as a result of human immunodeficiency virus infection (2 patients), and those receiving immunosuppressive agents (4 patients), were excluded. Figure 1 shows the flow chart of how the patients were included and classified into groups of high association (HA), medium association (MA), and low association (LA) with risks of relapse. The groups were defined as follows: HA group by the presence of both initial cavity and positive sputum smear/culture after two months of anti-TB treatment; MA group by the presence of one risk factor alone; and LA group by neither risk factor. A total of 51 patients completed follow-up and were included for final analysis. Pulmonary TB was diagnosed by CXR and clinical manifestations, plus a positive sputum culture. Chest computerized tomography was performed for the patients in the HA group to confirm the presence of cavitations in the lung parenchyma. The study protocol was approved by the Institutional Review Board of Kaohsiung Chang Gung Memorial Hospital, and informed consent was obtained from all patients.

2.1. Processing of sputum samples for AFB smears and mycobacterial cultures

Concentrated sputum smears were examined for acid fast staining bacilli (AFB) at diagnosis and after 2-month anti-TB treatment for each patient, using both the Ziehl-Nielsen and auramine-rhodamine stains. The results were reported using the grading scale of the International Union against Tuberculosis and Lung Disease. A laboratory certified for mycobacteriological diagnostics performed homogenization, decontamination, and cytocentrifugation before culture. The culture techniques used both solid (LJ) and liquid (MGIT) media following standard procedures [34,35].

2.2. CXR grading of disease

Standard posterio-anterior CXR were taken at diagnosis and after two and six months of anti-TB treatment in all enrolled patients. Text book grading for pulmonary involvement was adopted for disease assessment [36]. A minimal lesion was defined as slight-to-

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                    │  126 patients enrolled  │
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  ┌──────────────────────┐                    ┌──────────────────────────┐
  │ 12 NTM infections    │◄─────────────────►│ 2 HIV infection          │
  │ 15 poor drug         │                    │ 4 immunosuppressive      │
  │    compliance        │                    │    agents                │
  └──────────────────────┘                    └──────────────────────────┘
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                    │ 93 met inclusion criteria │
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  ┌──────────────────────┐                    ┌──────────────────────────┐
  │ 9 refused to sign    │◄─────────────────►│ 9 lost to follow up      │
  │    consent           │                    │ 2 MDR-TB                 │
  │ 12 inadequate blood  │                    │ 10 negative sputum       │
  │    samples           │                    │    culture               │
  └──────────────────────┘                    └──────────────────────────┘
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                    │ 51 completed follow up   │
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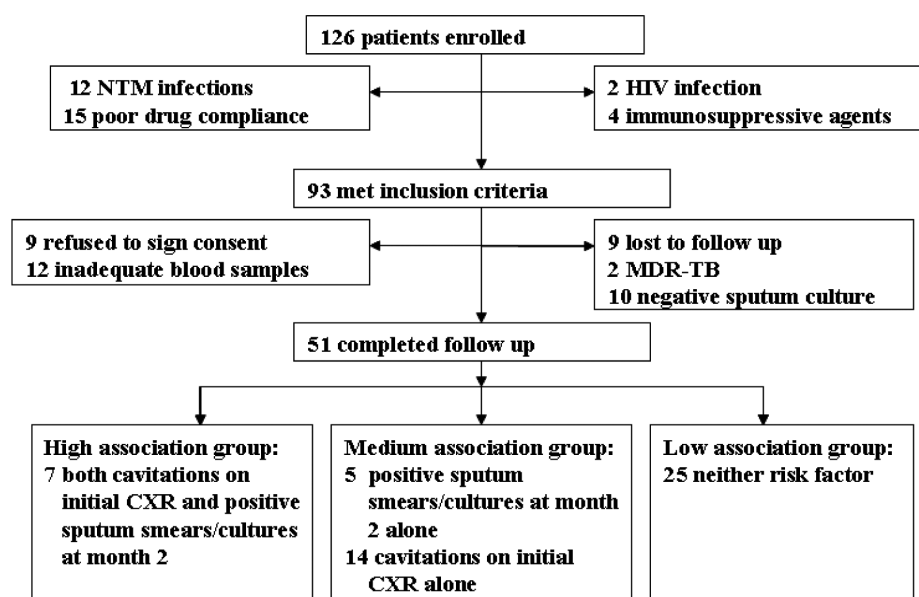| High association group:	Medium association group:	Low association group:
7 both cavitations on initial CXR and positive sputum smears/cultures at month 2	5 positive sputum smears/cultures at month 2 alone 14 cavitations on initial CXR alone	25 neither risk factor

Fig. 1. Flow chart to show classification profiles. M.tb = Mycobacterium tuberculosis; NTM = non-tuberculous mycobacterium; HIV = human immunocompromised virus; MDR = multidrug resistant.

moderate density not containing demonstrable cavitation with total extent not exceeding the lung volume on one side above the chondro-sternal junction. A far-advanced lesion was defined as slight-to-moderate density that extended more than the total volume of one lung or an equivalent in both lungs; the total diameter of the cavitation, if present, had to be more than 4 cm. Delayed resolution on CXR at month 2 was defined as a reduction of all the lesions by less than half after two months of treatment.

2.3. Treatment

All patients were treated in accordance with the American Thoracic Society guidelines for the management of TB [1]. Briefly, first line drugs were given in the following daily doses in combinations: Isoniazid (300 mg), Rifampin (10 mg/kg), Pyrazinamide (1–1.5 g), and Ethambutol (15–20 mg/kg). Second line drugs, including streptomycin and fluoroquinolones were substituted for one or more of the first line drugs in special situations such as drug intolerance or resistance. Every patient received direct observed treatment, short-course (DOTS) strategy and regular follow-up at our Pulmonary Clinic. Patients were managed for at least six months or longer depending on the presence of cavitations or extent on CXR, 2-month sputum smear/culture results, and clinical response to treatment.

2.4. Measurements of IP-10, IL-17, and CRP in the serum

IP-10 and IL-17 levels were assessed by a commercial ELISA method (R&D systems, Minneapolis, MN). All samples were assayed in duplicate according to the manufacturer's protocol. Hypersensitivity CRP (hsCRP) levels were measured by nephelometry (Dade Behring BM2, Marburg, Germany) according to standard procedures with a functional sensitivity of 0.18 mg/L. Blood samples at diagnosis within two weeks of anti-TB treatment (month 0) and after two months of anti-TB treatment (month 2) were obtained and analyzed from all patients (7 HA, 19 MA, 25 LA), and after six months of anti-TB treatment (month 6) from 35 patients (6 HA, 12 MA, 15 LA).

2.5. Statistical analysis

Continuous values were expressed as mean ± standard deviation (SD). The differences between the HR, MR, and LR groups were analyzed by Mann-whitney, Wilcoxon ranked sum, Kruskal-Wallis H, and chi-square tests, since they were not normally distributed. To allow random effects to be properly specified and to handle some missing data in the 6-month longitudinal experiments, mixed model analysis was used to compare blood levels between the HA and MA/LA groups at three different time points. To investigate predic-

Table 1
Comparison of the characteristics between high association (HA) and medium/low association (MA/LA) groups

Characteristics	HA group $N = 7$	MA/LA group $N = 44$	P value
Age, years	52.1 ± 15.0	57 ± 18.6	0.434
Male, n (%)	6 (85.7)	32 (72.7)	0.516
Co-morbidity, n (%)			
Diabetes mellitus	5 (71.4)	12 (21.3)	0.021
COPD	2 (28.6)	4 (8.9)	0.129
Chronic renal failure	0 (0)	5 (11.1)	0.354
Chronic hepatitis	0 (0)	1 (2.2)	0.69
Congestive heart failure	0 (0)	2 (4.4)	0.569
Malignancy	0 (0)	6 (13.3)	0.304
Smoking history, n (%)	6 (85.7)	22 (50)	0.078
Alcoholism, n (%)	2 (28.6)	10 (22.7)	0.735
Systemic symptoms, n (%)	6 (85.7)	27 (60)	0.189
Sputum at diagnosis, n (%)			
Smear AFB positive	6 (85.7)	32 (72.8)	0.446
1+	1 (14.3)	8 (18.2)	
2+	0 (0)	4 (9.1)	
3+	5 (71.4)	20 (45.5)	
Drug-resistant strain	2 (28.6)	8 (17.8)	0.5
CXR at diagnosis, n (%)			
Severity			0.577
Minimal lesions	0 (0)	4 (9.1)	
Moderately advanced	1 (14.3)	10 (22.7)	
Far advanced lesions	6 (85.7)	30 (68.2)	0.344
Bilateral involvement	6 (85.7)	31 (70.5)	0.401
Cavities	7 (100)	14 (31.8)	0.001
Pleural effusion	1 (14.3)	8 (18.2)	0.802
Time from starting treatment to collecting blood, days	3 ± 5.5	2.3 ± 4.5	0.825

COPD = chronic obstructive pulmonary disease; CXR = chest radiograph; AFB = acid fast bacilli.

tive accuracy, the candidate predictor was analyzed by area under the receiver operating characteristic (ROC) curves. Multivariate Cox proportional hazards regression analysis with stepwise forward selection was used to evaluate independent prognostic factors associated with survival, and age, smoking history, and month-2 serum IL-17 level with a cut-off value of 17 pg/ml were used as covariates. The Kaplan-Meier method was used to estimate overall survival. Differences in survival among groups were analyzed with the log-rank test. The null hypothesis was rejected at $p < 0.05$. Analyses were performed using the SPSS 15.0 statistical software package (SPSS Corp., Chicago, IL).

3. Results

3.1. Comparisons of clinical characteristics of patients between the HA and MA/LA groups

None of the serum protein levels measured in the 19 MA patients at any of the 3 time points differed from that of the 25 LA patients, so we lumped them together (MA/LA group). Baseline clinical characteristics of the 51 culture-positive TB patients at high and

medium/low risk of relapse are presented in Table 1. A larger proportion of patients in the HA group had diabetes mellitus than that in the MA/LA group ($p = 0.021$). Treatment responses based on microbiological and CXR findings, durations of individual medication and total treatment course, and all-cause mortality are presented in Table 2. A larger proportion of patients in the HR group had delayed resolution on CXR at month 2 ($p = 0.002$).

3.2. Higher serum IP-10 levels at month 2 in the HA group

There was a trend towards higher serum IP-10 levels ($p = 0.042$, Fig. 2A) for patients in the HA group throughout the 6-month treatment period by the mixed model analysis. In comparisons at individual time points, only month-2 IP-10 levels were significantly higher in the HA group than those in the MA/LA group after adjusting for diabetes mellitus (656.2 ± 234.4 vs. 307.6 ± 258.5 pg/ml, adjusted p value = 0.005). In contrast, serum IL-17 and hsCRP levels showed no difference between the HA and MA/LA groups (Fig. 2B and 2C). There was a trend towards lower serum hsCRP levels with time in all the patients during the 6-month

Table 2
Comparisons of treatment response, medication and outcomes between high association (HA) and medium to low association (MA/LA) groups with pulmonary tuberculosis

	HA group $N = 7$	MA/LA group $N = 44$	P value
Sputum at month 2, n (%)			
Smear positive	5 (71.4)	4 (10.3)	< 0.001
Culture positive	2 (28.6)	2 (4.5)	0.045
Smear or culture positive	7 (100)	5 (11.4)	< 0.001
Follow-up CXR findings, n (%)			
Delayed resolution at month 2	6 (85.7)	11 (25)	0.002
Extensive lesions at month 6	3 (42.9)	15 (35.7)	0.717
Duration of medication, months			
Isoniazid	8.1 ± 1.5	7.1 ± 2.6	0.291
Rifampin	8.1 ± 1.5	7.0 ± 2.5	0.253
Ethambutol	6.1 ± 2.9	6.1 ± 3.3	0.968
Pyrazinamide	2.7 ± 1	3.4 ± 2.6	0.497
Use of 2nd line medication*, n (%)	2 (28.6)	5 (11.4)	0.219
Treatment duration ⩾ 9 months, n (%)	5 (71.4)	25 (56.8)	0.466
All-cause mortality, n (%)	1 (14.3)	4 (7.3)	0.625**

CXR = chest radiograph; *Including Moxifloxacine, Levofloxacine, Ciproxine, and Streptomycin; **Compared by the log rank test.
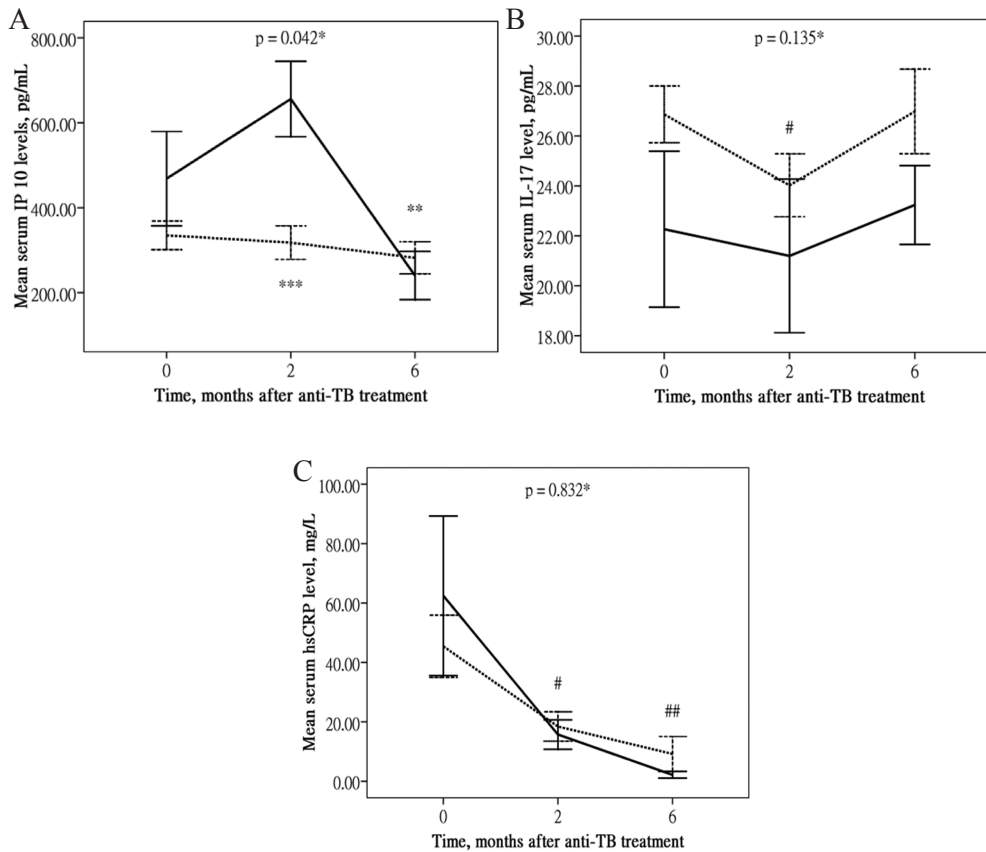
Fig. 2. Relationships between blood levels of IP-10, IL-17, and hsCRP, and risk of caviation and a positive sputum smear/culture after two months of anti-tuberculosis treatment in the study groups. Data are expressed as the mean values of IP-10 (A), IL-17 (B), and hsCRP (C) levels at 0, 2, and 6 months after treatment. Standard errors are indicated by vertical bars. Results of the medium association and low association (MA/LA) groups are shown by the dashed line, and results of the high association (HA) group are shown by the straight line. *Global p values for the fixed effect of grouping by the mixed model analysis; **$p < 0.05$, month 2 vs. month 6 for the HA group; ***$p = 0.005$, HA vs. MA/LA group at month 2; #$p < 0.005$, month 2 vs. month 0 for the MA/LA group; ##$p < 0.005$, month 6 vs. month 0 for the MA/LA group.
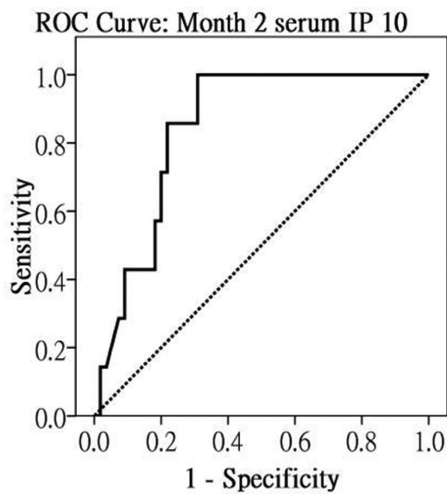
ROC Curve: Month 2 serum IP 10

Fig. 3. Predictive accuracy of the serum IP-10 levels measured at month 2. The corresponding ROC curve showed that the risk of relapse was well captured by month-2 IP-10 levels at a cut-off value of 431 pg/ml (AUC = 0.857, 95% CI 0.75–0.97, p = 0.003).

treatment period by the mixed model analysis (p = 0.001, Fig. 2C). In comparisons between two contiguous time points, serum hsCRP levels at month 6 were lower than that at month 0 (p = 0.002), and serum IL-17 levels at month 2 were lower than that at month 0 (p = 0.009) only in the MA/LA group. After six months of anti-TB treatment, patients in the HA group had a reduction in their serum levels of IP-10 (661.9 ± 256.2 vs. 240.4 ± 138.8 pg/ml, p = 0.028) and hsCRP (14.19 ± 12.95 vs. 2.14 ± 2.42 mg/L, p = 0.043) compared to those at month 2. The corresponding ROC curves showed that the risk of both cavitation and a positive sputum smear/culture at month 2 was well-captured by month-2 IP-10 levels at a cut-off value of 431 pg/ml (AUC = 0.857, 95% CI 0.75–0.97, p = 0.003), displaying a sensitivity of 85.7% and specificity of 79.5% (Fig. 3).

3.3. Correlations of serum IP-10/hsCRP levels at diagnosis with disease severity

Serum IP-10 levels at diagnosis were significantly higher (424.4 ± 236.6 vs. 251.3 ± 208.4 pg/ml, adjusted p value = 0.007)) in patients with high sputum smear grading (sputum AFB 2+ to 3 + at diagnosis, N = 30) than those with low sputum smear grading (sputum AFB 0 to 1+ at diagnosis, N = 21), after adjusting for age, CXR grading, and time from starting treatment to collecting blood by linear regression analysis (Fig. 4A). Serum hsCRP levels at diagnosis were significantly higher (63.2 ± 72.8 vs. 12.5 ± 26.4 mg/L,

adjusted p value = 0.024) in patients with far-advanced lesions on initial CXR (N = 36) than those with minimal and moderately advanced lesions (N = 15), after adjusting for a past smoking history of more than 10 pack-years, the presence of cavitations on CXR, and time from starting treatment to collecting blood by linear regression analysis (Fig. 4B).

3.4. Lower serum IL-17 levels at month 2 in non-survivors

In the MA/LA group, four patients died of pneumonia with sepsis, two during the course of anti-TB treatment, and two after completing anti-TB treatment. In the HR group, one patient died of an unknown cause after completing anti-TB treatment. Serum IL-17 levels at month 2 were significantly lower in non-survivors than survivors (15.7 ± 2.9 pg/ml vs. 24.6 ± 8.2 pg/ml, p = 0.001) (Fig. 5A). Figure 5B shows the Kaplan–Meier survival curves for the high and low month-2 IL-17 groups based on a cut-off level of $\leqslant$ 17 pg/ml. Patients with month-2 IL-17 levels of $\leqslant$ 17 pg/ml had significantly higher all-cause mortality than those with > 17 pg/ml (p = 0.007). Table 3 shows the unadjusted and adjusted Cox proportional hazards analyses. On a bivariate level, having an older age (hazard ratio (HR) 1.18, 95% CI 1.02–1.37), a past history of cigarette smoking > 10 pack-years (HR 5.81, 95% CI 1.33–25.38), and a lower month-2 IL-17 level $\leqslant$ 17 pg/ml (HR 11.25, 95% CI 1.25–101.06) were significantly related to mortality in patients with TB in our samples. Multivariate analysis demonstrated that old age (HR 1.54, 95% CI 0.96–2.48, p = 0.076) and a month-2 serum IL-17 level of $\leqslant$ 17 pg/ml (HR 42.57, 95% CI 1.56-1158.67, p = 0.026) were independently associated with mortality when adjusting for other covariates (Table 3).

4. Discussion

We undertook this study to determine candidate biomarkers that can identify TB patients at high risk of the presence of both cavitations on initial CXR and sputum smear/culture positivity after two months of anti-TB treatment. We found that serum IP-10 levels measured after two months of anti-TB treatment served as a biomarker to identify TB patients with both factors. Additionally, serum IL-17 levels measured after two months of anti-TB treatment served as a biomarker associated with risk of death.
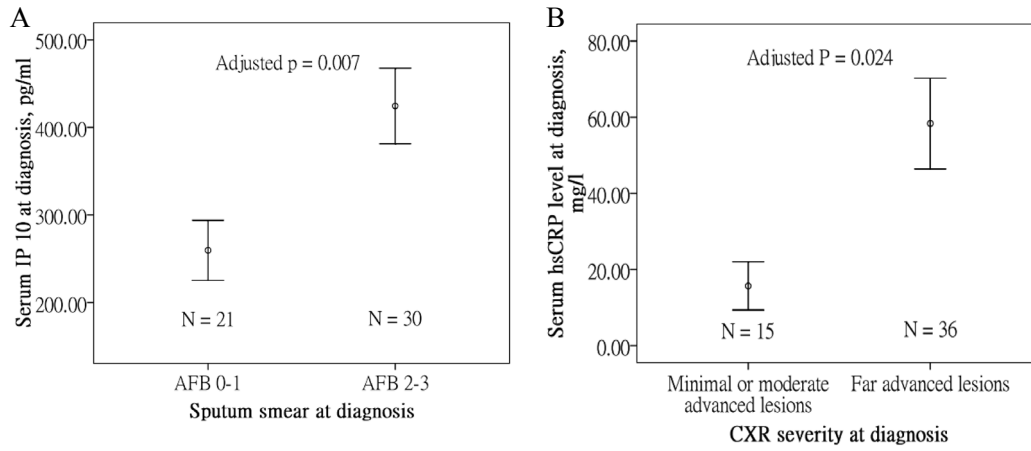
Fig. 4. Relationships between blood levels of IP-10 and hsCRP at diagnosis, and disease activity. (A) Serum IP-10 levels at diagnosis were significantly higher in patients with high sputum smear grading than those with low sputum smear grading. (B) Serum hsCRP levels at diagnosis were significantly higher in patients with far-advanced lesions on initial CXR than those with minimal to moderate-advanced lesions. Data are expressed as the mean values. Standard errors are indicated by vertical bars.
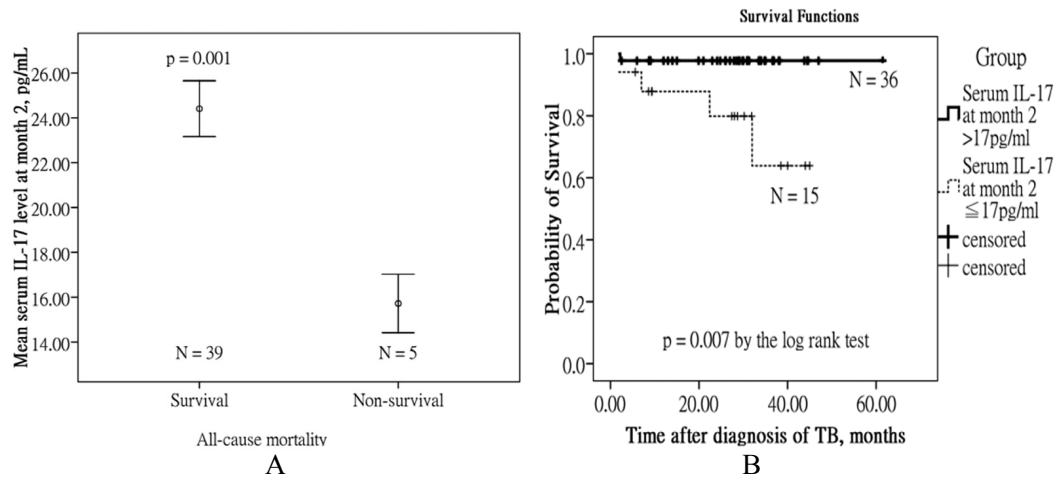

Fig. 5. Relationship between blood IL-17 levels measured at month 2 and all-cause mortality in the study population. (A) Serum IL-17 levels at month 2 were significantly lower in non-survivors than survivors. (B) Kaplan–Meier survival curves for the 51 TB patients with separate lines according to serum IL-17 levels at month 2 at a cut-off level of 17 pg/ml.

Serum IP-10 levels were elevated at month 2 in the HR group of patients, but did not differ during the 6-month treatment course in the MR/LR group of patients. Initial serum IP-10 levels also correlated with sputum smear grading but not CXR severity at diagnosis. This indicated that IP-10 may represent the burden of *Mycobacterium tuberculosis* in cavities and lung parenchyma. Accordingly, in a previous study by Azzurri et al., serum IP-10 levels were found to be correlated with the treatment efficacy but not clinical disease severity of pulmonary TB. They observed a significant decline in IP-10 levels after completion of anti-TB treatment in cured patients [17]. In another study by

Whittaker et al., plasma IP-10 levels were found to be even higher in patients with latent TB than those in patients with active TB [18]. IP-10 release from peripheral blood mononuclear cells is strongly induced after *M. tuberculosis* specific stimulation in patients with active and latent TB infections [19]. Taken together, human infection by *M. tuberculosis* may lead to an elevation of IP-10 levels, which can predict disease curability but not activity. In our study, IP-10 levels were not reduced at month 6 in the MA/LA group of patients. This may be contributable to a higher proportion of patients with far-advanced lesions at diagnosis and hence a higher

Table 3
Cox proportional hazard model to analyze predictors for mortality in all the patients with pulmonary tuberculosis

	Bivariate Hazard ratio	95% CI	Multivariate Hazard ratio	95% CI
Age	1.18	1.02–1.37*	1.54	0.96–2.48
Smoking history	5.81	1.33–25.38*		
Sputum AFB at month 0	0.9	0.47–1.76		
CXR grading at month 0	36.2	0.01–11368.3		
Co-morbidity	3.86	0.43–34.69		
Cavitation on initial CXR	0.98	0.16–5.85		
Positive smear/culture at month 2	0.98	0.11–8.75		
Cavitation and positive smear/culture at month 2	1.75	0.2–15.73		
Month-2 serum IL-17 $\leqq$ 17 pg/mL	11.25	1.25–101.06*	42.57	1.56–1158.67*

CI = confidence interval; CXR = chest radiograph. $*p < 0.05$.

proportion of patients with extensive lesions at month 6.

None of the patients who finished their anti-TB treatment course developed a clinically detectable relapse, except for one in the HA group who died of an unknown cause. Several factors may explain why relapses did not occur in our study patients. Firstly, 71.4% of the patients in the HA group and 56.8% of the patients in the MA/LA group received at least nine months of anti-TB treatment. Prolongation of the continuation phase from four to seven months has been demonstrated to be an effective method for reducing the relapse rate in previous studies [7,8]. Secondly, patients in both groups received EMB treatment for a mean duration of 6.1 months, rather than just for the initial consolidation phase of two months. Thirdly, all the patients received direct observed treatment as the internationally recommended strategy for TB control [37]. Furthermore, the proportion of patients who received second line medications was greater than 10%, and the proportion of patients who received at least nine months of treatment was greater than 50% in both the HA and MA/LA groups. This may reflect a higher disease severity in our study population, but good control of the infection under the extended regimens.

Another important finding in this study was that there were lower serum IL-17 levels in non-surviving patients. IL-17 can act to mediate the accumulation of polymorphic and mononuclear cells during mycobacterial infections. IL-17 mRNA levels were more prominently expressed in blood CD8+ T cells from patients with TB when compared to those from healthy subjects [30]. IL-17 levels have been linked to clinical outcomes in several diseases. IL-17-neutralized mice have exhibited significantly greater body weight loss, higher organism growth, and much more severe lung pathology with Chlamydia infection [28]. IL-17 expression has been demonstrated to be an independent

prognostic factor for survival in patients with non-small cell lung cancer [38]. Increased allergen-induced IL-17 responses during sublingual immunotherapy are associated with elevated symptom scores in allergic children [39]. For the first time, we found that IL-17 may play a role in predicting death in patients with pulmonary TB, although the sample size is relatively small for survival analysis. We speculate that lower serum IL-17 levels may reflect a weaker Th1 immune response against mycobacterial infections, since IL-17 is an important cytokine in the induction of optimal Th1 response, the maintainence of the integrity of the granuloma by limiting neutrophil death, and protective immunity against mycobacterial infection [26,27]. Further large scale studies are needed to confirm this association, and the underlying mechanisms by which lower serum IL-17 levels were linked to higher mortality remain to be further investigated.

The present study is biased and has several limitations. The sample size in the HR group was relatively small. However, we estimated the power to be 95.32% for the comparison of month-2 IP-10 between the HA and MA/LA groups, using the _nonparametric_ test formulations with a simple adjustment to the sample sizes in our study and an α error of 0.05 with PASS 2005 (NCSS, Kaysville, Utah, USA) software. Secondly, there was a large loss to follow up or exclusion in the study population. Some patients were transferred to local physicians or other hospitals because of their preference or convenience. Some patients refused to provide blood samples at the 2nd or 3rd time point. Patients with multidrug resistant (MDR)-TB or negative sputum culture were excluded because drug-resistant strains may potentially influence the levels of inflammatory cytokines, and negative culture may raise the uncertainty of clinical diagnosis. Finally, a larger proportion of patients in the HR group had diabetes mellitus. Additionally, some blood samples were not col-

lected before starting anti-TB treatment, and this would potentially affect the cytokine levels since changes in immune responses might occur as early as 1 week post treatment. However, we have made statistical adjustments for these confounding factors by linear regression analysis.

In conclusion, serum IP-10 levels measured after two months of anti-TB treatment may be a biomarker for estimating the risk of cavitation and a positive sputum smear/culture after 2-month treatment in patients with pulmonary TB, and can be helpful in making an individual-based decision on preventive and therapeutic treatment schemes. In addition, serum IL-17 levels measured after two months of anti-TB treatment may be a biomarker for predicting long-term survival.

5. Competing interests

All authors of this paper declare that there is no conflict of interest in this study or in reporting the findings described in this manuscript. No specific funding has been received by any author.

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