Decreased Osteopontin Expression as a Reliable Prognostic Indicator of Improvement in Pulmonary Tuberculosis: Impact of the Level of Interferon-γ-Inducible Protein 10

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Key Words
Osteopontin • IP-10 • CRP • Biomarkers • Tuberculosis

Abstract
Background/Aims: Osteopontin (OPN) expression is increased during the course of various chronic inflammatory diseases, including tuberculosis (TB). However, its prognostic value in TB management remains unclear. This study aimed to determine whether OPN could associate with other cytokines serving as a reliable biomarker for evaluating the effectiveness of early anti-TB treatments.

Methods: Smear-positive pulmonary TB patients (n = 20) were recruited, and the plasma levels of OPN, IP-10, TNF-α, and IL-12 were measured by ELISA before initiation of anti-TB therapy and after sputum smear conversion. The C-reactive protein (CRP) levels and erythrocyte sedimentation rate (ESR) were also tracked during anti-TB treatment.

Results: OPN expression was significantly elevated in patients with smear-positive pulmonary TB, and was closely related with disease severity. Monitoring during the treatment course revealed that its expression, along with that of IFN-γ-induced protein 10 (IP-10), decreased significantly only after sputum smear conversion. Moreover, OPN levels positively correlated with CRP levels before and after anti-TB treatment. Furthermore, OPN markedly promoted IP-10 expression in peripheral blood mononuclear cells.

Conclusion: Association between OPN and IP-10 may serve as a reliable prognostic indicator for improvement during the early treatment of pulmonary TB, and may help clinicians in tailoring an effective TB treatment regimen.

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Introduction

Tuberculosis (TB), a chronic lung disease caused by Mycobacterium tuberculosis (MTB), ranks among the top ten deadliest communicable diseases in low and middle-income countries. In 2013, there were 9.0 million new cases of TB diagnosed and 1.5 million deaths [1]. With improvements in diagnosis and treatment, the disease rate is slowly declining each year, and it is estimated that 37 million lives have been saved in recent decades [1]. Though anti-mycobacterial peptides and new antibiotics have a few advantages during TB treatment [2, 3], the treatment efficacy has been considerably compromised by the emergence of multidrug-resistant TB (MDR-TB) strains. In order to enable rapid treatment and disease control, it is necessary to strengthen surveillance during the treatment course. To this end, the identification of biomarkers that could serve as prognostic factors for improvement during treatment is crucial.

The expression of osteopontin (OPN), a multifunctional phosphorylated glycoprotein associated with inflammation and tissue repair, is elevated in certain chronic inflammatory diseases, particularly tuberculosis [4, 5]. This glycoprotein is produced by macrophages, T cells, and natural killer cells, and its expression is upregulated following infection with MTB and other pathogens [4, 6]. OPN is of interest in TB as it can, via the enhancement of IL-12 and IFN-γ secretion [4, 7], polarize the immune response towards a T helper 1(Th1)-type, and such responses are now believed to play a key role in lung pathogenesis following MTB infection. A substantial increase in OPN expression was observed following infection of a macrophage cell line with MTB [8], and OPN-deficient mice showed reduced clearance of Mycobacterium bovis bacillus Calmette-Guérin (BCG) and increased granuloma formation [9]. In addition, patients suffering from diverse granulomatous diseases including tuberculosis displayed pulmonary OPN expression in association with the granuloma [10]. The plasma levels of OPN have been measured in patients with pulmonary tuberculosis, and were found to be generally higher than those in healthy controls [11]. Additionally, the OPN levels paralleled the extent of lung lesions and the degree of fever in patients with pulmonary TB, and could be useful to evaluate activity of TB disease [11]. However, we attempted to explore OPN value in sputum smear conversion period of pulmonary TB patients in our study.

IFN-γ-induced protein 10 (CXCL10/IP-10), a member of the α-chemokine subfamily, is predominantly expressed during the Th1-mediated pathological inflammatory response, and is involved in multiple biological processes, including apoptosis induction, cell growth inhibition, and the recruitment of activated T-cells, macrophages, and NK cells to the site of infection [12]. High levels of IP-10 are detected in the serum of TB patients [13], and the level of this chemokine and the number of chemokine-positive cells are both higher in the bronchoalveolar lavage fluid of this group compared to healthy controls [14]. IP-10 production is also significantly augmented in mice following exposure to low-dose MTB aerosols [15]. In addition, serum levels of IP-10 are higher in acute respiratory distress syndrome patients with a fatal outcome than in survivors, suggesting that high levels of IP-10 are associated with disease severity in this condition [16]. A report showed IP-10 could significantly differentiate active TB from the LTBI group, irrespective of HIV status and declined gradually during anti-TB chemotherapy [17]. However, whether there was a correlation between changes of IP-10 level and sputum smear conversion remains to be thoroughly understood.

In this study, we showed that OPN levels were associated with disease severity, and that both OPN and IP-10 levels were elevated in patients with smear-positive pulmonary TB. Therefore, we prospectively tracked changes in the OPN and IP-10 levels during pulmonary TB treatment and sought to determine whether OPN could induce the expression of IP-10, and whether association between OPN and IP-10 could serve as a reliable prognostic indicator for improvement during the early treatment of pulmonary TB.
Materials and Methods

Patients and associated procedures

All study subjects were recruited at the First Affiliated Hospital College of Medicine, Zhejiang University in the June 2014-November 2014 period. Twenty smear-positive TB patients and 10 age- and gender-matched healthy individuals recruited within the same period were selected as study subjects. In the TB group, 10 patients were confirmed to have pleural effusion on the basis of chest X-ray and chest CT. Diagnosis of all tuberculosis cases included in this study was done in accordance with China's TB diagnosis standard, and infections due to other pathogens or autoimmune diseases were ruled out in each case. The study design was approved by the Ethics committee of the First Affiliated Hospital College of Medicine, Zhejiang University, and written informed consent was obtained from all patients and healthy volunteers. Clinical data and physical examination findings for all enrolled patients are shown in Table 1.

Sample Collection

Blood samples (3-mL samples) were collected in heparinized tubes at the time of diagnosis and before initiation of anti-tuberculosis therapy. Another sample was collected 2-8 weeks after therapy initiation when symptomatic improvement and AFB smear conversion were observed. Plasma was separated from peripheral blood mononuclear cells (PBMCs) using density gradient centrifugation, and was stored at -80°C until use, while the PBMCs were used in cell culture experiments.

| Table 1. Clinical characteristics of patients with pulmonary tuberculosis. 1) HERZ, isoniazid, ethambutol, rifampicin, plus pyrazinamide. Categorical variable data are presented as positive/tested (%). Continuous variable data are shown as the mean ± SD (range) |
|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Patient characteristics | Patients with smear-positive pulmonary tuberculosis (n=20) | Patients with tuberculous pleurisy (n=10) |
|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Age (years) | 55±19.8 (18-86) | 45±21.9 (20-83) |
| Sex (m/f) | 13/7 | 5/5 |
| Symptoms | | | |
| Fever | 37.7±0.87 | 37.8±0.93 |
| Sweat | 6/20 (30) | 2/10 (20) |
| Cough | 11/20 (55) | 2/10 (20) |
| Loss of appetite | 4/20 (20) | 3/10 (30) |
| Underlying medical disorders | 2/20 (10) | 1/10 (10) |
| Radiographic features | | | |
| Infiltration | 20/20 (100) | 10/10 (100) |
| Cavitation | 7/20 (35) | - |
| Effusion | 3/20 (15) | 10/10 (100) |
| Smear grading | | | |
| 4+ | 5/20 (25) | - |
| 3+ | 6/20 (30) | - |
| 2+ | 5/20 (25) | - |
| 1+ | 4/20 (20) | - |
| Laboratory findings | | | |
| WBC (x10⁹/L) | 6.7±4.0 | 5.1±1.8 |
| Neutrophil | 4.1±1.8 | 3.5±1.5 |
| Lymphocyte | 1.2±0.5 | 0.9±0.5 |
| Monocyte | 0.57±0.19 | 0.50±0.34 |
| Eosinophil | 0.10±0.07 | 0.08±0.07 |
| Basophils | 0.03±0.02 | 0.02±0.01 |
| CRP (mg/L) | 35.5±31.5 | 32.2±29.8 |
| ESR (mm/h) | 43.9±18.9 | 33±21.5 |
| Resistance | - | - |
| Treatment | | | |
| HERZ | 20/20 (100) | 4/10 (40) |
| Other than HERZ | - | 6/10 (60) |
Cell culture

PBMCs and human lung epithelial cells of the A549 cell line were propagated at 37°C, 5% CO₂ in RPMI 1640 (Life Technologies, USA) or DMEM (Life Technologies, USA), respectively, supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% fetal bovine serum (FBS) (Life Technologies, USA). Cells were seeded in 96-well tissue culture plates at 2×10⁶ cells/mL. For A549 cell culture, following 60-70% adherence, 1 μg/mL recombinant osteopontin (rOPN; R&D, USA) was added, and supernatants were harvested after a 24-h incubation and assayed for IP-10. For PBMC culture, cells were incubated with 1 μg/mL recombinant OPN, and cell culture supernatants were harvested at 24 h and assayed for IL-12, INF-γ and IP-10 by ELISA. In parallel experiments, cells were pretreated for 2 h with neutralizing anti-IFN-γ monoclonal antibody (10 μg/ml) (R&D, USA), and then recombinant osteopontin was added (1 μg/mL).

ELISPOT assay

According to the manufacturer’s instructions, IFN-γ ELISPOT assay was performed using commercially available kits (eBiosciences, USA). PBMCs were cultured with 1 μg/mL recombinant OPN for 24 h, and the positive cells enumerated by an ImmunoSpot S5 Macro Analyzer (C.T.L., Shaker Heights, OH, USA) were expressed as numbers of IFN-γ spot-forming units per well.

LDH assays

A549 cells were seeded in 96-well tissue culture plates at 2×10⁶ cells/mL, following 60-70% adherence, 1 μg/mL recombinant osteopontin (rOPN; R&D, USA), 15 μg/mL BCG and 100 ng/mL IFN-γ (R&D, USA) were added respectively for 24 h, and then determine the concentrations of LDH (Roche, Mannheim, Germany) according to the manufacturers’ instructions. In parallel experiments, cells were treated with both IFN-γ (100 ng/mL) and OPN (1 μg/mL) or BCG (15 μg/mL) for 24 h, and then the concentrations of LDH were detected.

ELISAs

ELISA kits for OPN (from R&D, USA) and TNF-α, IP-10, IFN-γ and IL-12 (all from eBioscience, San Diego, CA, USA) were used to determine the concentrations of the biomolecules, according to the manufacturers’ instructions.

Statistical analyses

Data are presented as the mean ± SD, and were analyzed using the Student t-test or the paired data t-test. Correlation analysis was carried out using the Pearson correlation coefficient. Differences where P < 0.05 were regarded as statistically significant.

Results

High plasma OPN concentrations are related with the severity of TB

To investigate the pathogenic role of OPN in tuberculosis, we compared plasma OPN levels in patients with smear-positive pulmonary TB and that in healthy control subjects. As shown in Fig. 1A, plasma OPN concentrations were significantly (p < 0.001) increased in patients with smear-positive pulmonary TB. The immune response to MTB can damage lung tissue, producing defects that appear as cavities. In this study, lung cavitation in patients was confirmed by chest X-ray and CT, and the plasma OPN levels in patients with or without cavitation were examined; the OPN levels were significantly elevated (p < 0.05) in patients with cavitation (Fig. 1B). Similarly, the OPN levels were significantly increased (p < 0.05) in patients who had fever compared to those who did not (Fig. 1C). In addition, patients with tuberculous pleural effusion (TPE), a common form of extrapulmonary TB involving a delayed hypersensitivity reaction and fluid accumulation in the pleural space following release of antigenic MTB proteins into the pleural cavity [18], showed significantly higher levels of OPN (p < 0.01) than healthy controls (Fig. 1D). Furthermore, the OPN levels in TPE patients were higher than those in smear-positive pulmonary TB patients. TPE patients who presented with fever also showed increased OPN levels (p < 0.05) (Fig. 1E), which was
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Increased expression of IP-10, TNF-α, and IL-12 in TB

Similar to the results of patients with smear-positive pulmonary TB. In addition, we treated human alveolar epithelial cells for 24 h with BCG, and found a significant increase (p < 0.01) in the expression of OPN (Fig. 1F). Collectively, these findings indicate that OPN expression is increased during TB, and this increase appears to be associated with disease severity.

**Fig. 1.** Increased OPN levels in patients with TB. Plasma OPN levels in smear-positive pulmonary TB patients (A) and TPE patients (D) were measured by ELISA, and compared to that in healthy controls (HC). The plasma OPN levels were compared in smear-positive pulmonary TB patients (B) with or without cavitation. OPN concentrations in smear-positive pulmonary TB patients (C) and TPE patients (E) with or without fever. (F) OPN levels in culture supernatants of A549 cells incubated with BCG (15 μg/mL) for 24 h. Data represent the mean ± SD. *, P < 0.05. **, P < 0.01. ***, P < 0.001.

**Fig. 2.** Increased expression of chemokines and cytokines in patients with TB. IP-10 (A), TNF-α (B), and IL-12 (C) levels in the plasma of smear-positive pulmonary TB patients and healthy individual controls were compared before treatment by ELISA. The plasma levels of IP-10 (D), TNF-α (E), and IL-12 (F) in TPE patients were detected. Data represent the mean ± SD. *, P < 0.05. **, P < 0.01. ***, P < 0.001.
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During pulmonary infection. We examined the expression of IP-10, TNF-α, and IL-12, which collectively indicate the degree of inflammation, in patients with smear-positive pulmonary TB and in healthy controls, and found an increase in the plasma IP-10, TNF-α, and IL-12 levels in the patient population (Fig. 2). Similar results were observed in patients with TPE (Fig. 2).

**Decrease cytokine and chemokine levels following sputum smear conversion**

A previous study suggests that most patients with smear-positive pulmonary TB become smear-negative after 2 weeks of appropriate treatment [20]. Persistent smear positivity after 2 months of treatment is predictive of an unfavorable outcome such as treatment failure.
In our study, all patients with smear-positive pulmonary TB became smear-negative within 8 weeks of an appropriate treatment. Patients were then divided into two groups based on the time of sputum smear conversion. Group 1 included patients whose sputum smear conversion occurred during the first 2 weeks of treatment, while group 2 consisted of patients who became smear negative during 2-8 weeks of treatment. We tracked the plasma levels of OPN, IP-10, TNF-α, and IL-12 at different time points in the patients of group 1 (at the start of the treatment and 2 weeks) and group 2 (at the start of the treatment, 2 weeks, and 8 weeks) (Fig. 3). We found that while TNF-α and IL-12 levels decreased steadily during treatment in both groups (Fig. 3A and B), interestingly, the OPN and IP-10 levels decreased significantly only after sputum smear conversion in both groups; an examination of the group 2 levels indicated that in patients who had slower sputum smear conversion, the levels were not significantly different by the second week of treatment, unlike that in the group 1 patients (Fig. 3C and D).

Changes in CRP level and the ESR after sputum smear conversion

CRP and the ESR are considered to be indicators of inflammation, and both parameters are increased in the acute phase of pulmonary TB [22]. In our study, CRP levels and the ESR were found to be elevated in patients with smear-positive pulmonary TB, and a decline was observed as the treatment progressed (Fig. 4). However, while the ESR values declined steadily as the treatment progressed (Fig. 4A), a significant decrease in CRP levels was observed only after sputum smear conversion, and there was no significant decrease in CRP levels by week 2 in the group 2 patients, unlike that in group 1 (Fig. 4B).
Correlations between plasma OPN levels and CRP or IL-12 in patients with TB before and after anti-TB treatment

Given that OPN and CRP were significantly elevated in smear-positive pulmonary TB patients, we attempted to determine a relationship between the before and after anti-TB treatment. The plasma OPN levels were found to be positively correlated with CRP levels both before (R = 0.7873, P < 0.0001) and after (R = 0.7467, P < 0.001) anti-TB treatment (Fig. 5A and B). It has been reported that IL-12 can mediate Th1 responses in pulmonary TB, and that OPN can induce the production of IL-12 [4]. In support of this, our analysis indicated a positive correlation between plasma OPN levels and IL-12 levels both before (R = 0.7518, P < 0.001) and after (R = 0.7168, P < 0.01) sputum smear conversion (Fig. 5C and D).

OPN treatment increases the production of IP-10

To examine the relationship between OPN and IP-10, we analyzed the correlation of OPN and IP-10 in smear-positive pulmonary TB patients both before and after sputum smear conversion and found a positive correlation between plasma OPN levels and IP-10 levels (Fig. 6A and B). The similar result was observed in TPE patients before treatment (Fig. 6C). And on this basis, we speculated whether OPN treatment could induce the production of IP-10 in human alveolar epithelial cells and PBMCs. Both cell types were stimulated with OPN, and an assay of culture supernatants at 24 h revealed that IP-10 production was significantly augmented by OPN treatment (Fig. 7A and B). It has been reported that OPN involved in the pathologic process of active TB by inducing IL-12-mediated Th1 cell responses [23], and that OPN can induce the expression of IFN-γ [4]. In our study, OPN could augment the production of IL-12 and IFN-γ in both PBMCs from TB patients and health controls (Fig. 7C and D). Furthermore, ELISPOT assays showed that the numbers of IFN-γ secreting cells were significantly increased in OPN stimulation group (Fig. 7E). In addition, when IFN-γ induced by OPN was neutralized by IFN-γ antibody, the production of IL-12 caused by OPN stimulation was suppressed (Fig. 7F). Our data indicates that OPN expression correlates with disease severity and lung damage (Fig. 1). To further confirm these results, we examined whether OPN or BCG treatment of human alveolar epithelial cells affected the release of LDH, a cytosolic enzyme that is released upon cell damage or lysis [23]. Indeed, stimulation with either OPN or BCG increased LDH release by human alveolar epithelial cells (Fig. 7G). In addition, we found IFN-γ could enhance the LDH release, and LDH level induced by both IFN-γ and OPN was higher than by OPN alone in human alveolar epithelial cells (Fig. 7G). These results further supported that OPN can aggravate lung damage directly, and indirectly through prompting the IFN-γ secretion of PBMCs.
Discussion

Herein, our findings indicate that OPN, which is associated with inflammation and tissue repair, is elevated in the plasma of patients with smear-positive TB, and that its expression is also positively correlated with disease severity, and decreases concomitantly with an improvement in clinical parameters. These findings are supported by previous reports, which showed that stimulation of human alveolar macrophages with MTB caused an appreciable increase in OPN expression [4], and that OPN levels were higher in TB patients than in control subjects, and correlated well with severity of pulmonary TB [4, 11]. The latter finding is supported in our study as well; plasma OPN concentrations in the smear-positive TB patient population correlated with parameters of disease severity, such as cavitation, fever, and TPE. These data also suggest that OPN may be involved in TB pathogenesis. A report indicated that the early immune response to M. tuberculosis infection was not affected to a major extent by OPN deficiency, whereas OPN may even be detrimental for the host during the late phase.
of tuberculosis [24]. So tracing changes of OPN is very important during recovery following the treatment initiation.

CD4\(^+\) Th1 lymphocytes are required for an effective acquired immune response against MTB [25], and Th1-linked chemokines and cytokines may also be involved in disease pathogenesis [26]. IL-12 production by dendritic cells recruited to the site of infection forms an essential early step and triggers the Th1 response [27]. The IFN-γ-induced IP-10 recruits T cells to sites of inflammation and also contributes to the necrosis of tuberculous granulomas by inhibiting angiogenesis [28]. TNF-α, which is expressed in MTB-infected tissues, can influence granuloma organization and alsonmodulate macrophage function [29, 30]. We found that patients with smear-positive pulmonary TB had higher plasma levels of IP-10, TNF-α, and IL-12 than healthy subjects. Similar results were observed in patients with TPE. Whereas, in a study, there were no significant differences in IL-12 levels of patients with pulmonary tuberculosis and miliary tuberculosis compared with healthy controls [11]. We considered different grouping criteria led to the conflicting results, as in our study, we just recruited patients with smear-positive TB, while patients were divided into minimal, advanced and far advanced TB, irrespective of sputum smear status in their research. Our findings indicate that the expression of IP-10, TNF-α, and IL-12 increases dramatically during the acute inflammatory period of TB, which were supported by previous reports [25, 26]. However, changes in the expression of these soluble mediators following the initiation of treatment have not been well explored in smear-positive pulmonary TB patients.

Successful control of TB requires an early and effective control of MTB transmission from infectious individuals [31], with patients with smear-positive pulmonary TB being highly infectious. The number of acid-fast bacilli (AFB) decreases rapidly after the initiation of anti-TB treatment. Sputum smear conversion indicates that most of the AFB have been eliminated, and is associated with a reduction in the rate of treatment failure and relapse [32]. Many factors can influence sputum smear conversion, and hence it is necessary to monitor multiple parameters during treatment. Most patients with smear-positive pulmonary TB become smear negative within 2 weeks of an appropriate treatment [5]. Persistent smear-positivity after 2 months of treatment is predictive of an unfavorable outcome such as drug resistance and treatment failure [6]. In our study, all patients with smear-positive pulmonary TB converted to a smear-negative state within 8 weeks of treatment initiation. However, patients in this study could be divided into two groups depending on when smear conversion occurred, with one group converting rapidly, within the first 2 weeks of treatment, the other group with slower conversion from 2 weeks to 8 weeks of treatment. During treatment, dramatic changes in the OPN, IP-10, TNF-α, and IL-12 levels were observed. IP-10 level decreased more than 300pg/ml between 0 and 7 days of treatment [33], while our data showed a decrease in IP-10 level reached to over 500pg/ml between 0 and 2 week of group 2. In addition, we found while the levels of TNF-α and IL-12 declined steadily during treatment in group 1, those of OPN and IP-10 decreased significantly only after sputum smear conversion in group 2, and though OPN and IP-10 levels were slightly lower at 2 weeks already in group 2, which indicated that TB patients were sensitive to anti-tuberculosis therapy and the therapy was effective. Sputum smear conversion could determine treatment efficacy, and monitoring changes of OPN, IP-10 or CRP levels has more advantages in treatment efficacy before sputum smear conversion. Then we examined whether there is a relationship between OPN and IP-10, and whether OPN could serve as reliable prognostic indicator of improvement in association with IP-10 during early treatment.

Both CRP and ESR are established prognostic indicators in patients with pulmonary TB; CRP is an acute phase indicator of inflammation that reflects the disease severity [34], while ESR is regarded as an indicator of disease activity, and is also reflective of disease severity [35]. In agreement with the literature, CRP levels and the ESR were elevated in patients with sputum positivity in this study. However, the ESR decreased steadily as the treatment progressed, while the CRP declined dramatically only after sputum smear conversion.
Though CRP is a prognostic indicator, it is not specific in TB disease. So we need to seek another indicator to better monitor the condition changes. Interestingly, the changes in OPN levels during treatment showed a similar trend. Indeed, our analysis indicated a positive correlation between OPN and CRP levels in patients with smear-positive pulmonary TB.

OPN expression was found to be elevated in tissue sections from patients with various granulomatous diseases, including pulmonary tuberculosis [10, 36]. In addition, OPN may contribute to disease pathogenesis in pulmonary TB by triggering Th1-mediated inflammatory cascades [4]. Furthermore, patients undergoing chemotherapy show a drop in plasma OPN levels in parallel with clinical improvement, and OPN can be useful in determining expansion of active TB lesions [37]. In agreement with these studies, OPN levels sharply decreased only when sputum smear conversion occurred in our patient cohort. As sputum positivity is an indirect measure of disease activity, our findings support that OPN may be a hallmark of improvement during the treatment of pulmonary TB. However, we considered a single biomarker is not enough to assess the treatment effect. In order to find a reliable indicator, we further researched IP-10 and found there was a positive correlation between OPN levels and IP-10 levels both before and after anti-TB treatment.

An increase in IP-10 levels is observed in certain autoimmune disorders as well as acute coronary syndrome and allergy [38-40]. IP-10 is also secreted at high levels by monocytes and polymorphonuclear granulocytes following MTB infection [41]. High plasma IP-10 levels have been reported in TB patients, and were found to be associated with fever [13]. Thus, IP-10 is regarded as a promising candidate biomarker in MTB infection, even in HIV-infected individuals [42]. In this investigation, the levels of IP-10 also dramatically decreased only after sputum smear conversion, similar to the trend observed for OPN. This led us to investigate whether OPN may be involved in IP-10 synthesis, and we found that OPN could induce IP-10 expression not only in alveolar epithelial cells, but also in PBMCs. It has been reported that OPN involved in the pathologic process of active TB by inducing IL-12-mediated Th1 cell responses [23], in agreement with that OPN augmented the production of IL-12 in PBMCs in our study. IFN-γ triggers initiation of the major effector mechanism for the Th1 immune response in pulmonary TB [43] and is important in the cytokine cascade against MTB infection. We found the levels of IFN-γ and the numbers of IFN-γ secreting cells are both higher in OPN stimulation group compared to control group, and the production of IP-10 caused by OPN stimulation was suppressed with neutralizing IFN-γ induced by OPN. In other reports, the expression of both OPN and IP-10 is concurrently increased during the acute phase of MTB infection [44]. OPN levels correlate well with disease severity, while IP-10 levels correlate with the extent of inflammation [4, 45]. Moreover, OPN or IFN-γ could enhance the LDH release (indicative of cell damage) from alveolar epithelial cells and IFN-γ increased the release of LDH induced by OPN or BCG, which further supported that OPN can aggravate lung damage through directly or indirectly releasing LDH. Monitoring changes of OPN, IP-10 levels not only assess the effectiveness of the treatment, but also evaluate the degree of lung injury. So association between OPN and IP-10 may be served as a reliable indicator and could help gauge the response to a treatment course.

In conclusion, the present study, which shows a significant upregulation of OPN in patients with pulmonary TB, a strong correlation with various parameters of disease severity, and decrease in expression only after smear conversion suggests a possible contribution of OPN to the pathologic processes underlying pulmonary TB. This is also supported by the observation that changes in its expression were similar to that of IP-10, and moreover, that OPN could induce IP-10 expression. Taking these findings in combination with the observation that changes in OPN and IP-10 expression paralleled those of CRP, an established prognostic indicator in pulmonary TB, our study suggests that association between OPN and IP-10 may serve as a reliable prognostic indicator for notable improvement during the early treatment of pulmonary TB, and may help clinicians tailor and modify treatment regimens in a timely fashion.
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Disclosure Statement

The authors declare that they have no conflict of interest.

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