

Correlation Between Serum Tumor Necrosis Factor Alpha Levels and Clinical Severity of Tuberculosis

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This study verified the correlation between the serum levels of TNF alpha and different clinical forms of tuberculosis. We described a group of 24 patients presenting several clinical forms of tuberculosis and a control group of 13 healthy individuals. The levels of TNF alpha were measured by bioassay method. The levels of TNF-alpha had significant differences between the tuberculosis and control groups. The patients with abnormal chest X-Ray findings had higher TNF alpha levels (15328.48 ± 4602.19 pg/mL) when compared to patients with normal X-Rays (3353.18 ± 1495.29 pg/mL) ($p < 0.05$). Patients that lost weight had higher TNF alpha levels (15468.54 ± 4580.54 pg/mL) than those that didn't lose weight (2904.98 ± 1367.89) ($p < 0.05$). The levels of TNF alpha were higher in patients with a positive PPD skin test than in those with a negative PPD test ($p < 0.05$). There was a positive correlation between patients' clinical severity and the serum levels of TNF alpha. In patients with successive measurements of TNF alpha, we observed that there was a drop in cytokine levels, and also a clinical improvement concomitantly. We concluded that there was a correlation between serum TNF alpha levels and chest X-Ray alterations, loss of weight, positive PPD skin test and clinical severity in patients with tuberculosis. There was evidence of a worse clinical outcome in patients with tuberculosis that presented higher TNF alpha serum levels.

Key-Words: TNF alpha, tuberculosis, cytokines, clinical severity.

Abbreviations: TNF=tumor necrosis factor, TB=tuberculosis, *M. tuberculosis*=*Mycobacterium tuberculosis*, IL=interleukin, CTLs=cytotoxic T lymphocytes, MHC=major histocompatibility complex, IFN=interferon, BAL=bronchoalveolar lavage, CT=computed tomographic, ADA=adenosine deaminase.

The pathogenesis of the inflammatory response in tuberculosis (TB), involving mainly cellular and molecular phenomena, remains unclear. Macrophage activation, phagocytosis mechanisms, and the survival of *Mycobacterium tuberculosis* in phagocytic vesicles must be further studied.

When tuberculosis infection occurs, a variety of chemokines and cytokines are secreted from infected cells and tissue macrophages. TNF alpha increases early in the disease and takes part in the pathogenesis and prevention of mycobacterial infection. TNF alpha also appears crucial for the formation of *M. tuberculosis*-constraining granulomas, infection control and elimination of mycobacteria [1]. The production of TNF alpha in HIV-TB co-infected patients is down-regulated in T lymphocytes, and this may explain the cause of defective granuloma formation in this condition [2]. Serum levels of TNF alpha and IL-1 are proportional to the degree of tissue infection [3] and are also associated with induction of fever and other consumptive manifestations of the disease [4].

The cellular immune response is essential for TB resistance, mainly through cytokine production such as IL-

12, IL-18, IFN gamma, IL-2 and TNF alpha. The TCD8⁺ cells (CTLs) perform cytotoxic actions, mainly through major histocompatibility complex (MHC) class I molecules that, in association with CD1d molecules, present glycolipid antigens to T lymphocyte cells [5]. CTL cells may have a protective role in TB disease due to several mechanisms: 1) producing potent antibacterial cytokines such as IFN gamma and TNF alpha; 2) recognizing *M. tuberculosis* antigens presented by MHC associated to HLA-I complex; 3) inducing apoptosis in infected cells [6,7].

The role of cytokines produced by Th1/Th2 lymphocytes is complex due to the action of IL-12, IFN gamma, IL-4 and IL-10 (pro-inflammatory and anti-inflammatory cytokines). Several aspects of this complex interaction have been studied in recent studies: a) IFN gamma inhibition by IL-10 [8]; b) IFN gamma and IL-10 production stimulated by IL-12 [9]; c) IFN gamma and IL-10 production stimulated by TCD4⁺ clones in the bronchoalveolar lavage (BAL) fluid of patients with pulmonary TB [10]; d) Th2 cytokine association with histopathologic alterations in patients with TB [11].

Several studies have shown physiopathologic correlation between serum TNF alpha and the evolutive course of mycobacteria infection. TNF alpha neutralization caused an increase in pulmonary bacterial load in mice that had been infected with *M. tuberculosis* six months before [12]. There is an association between TNF alpha production and *M. tuberculosis* virulence in human monocytes, as well as a correlation between the increase in serum TNF alpha levels and the clinical deterioration in patients with a severe form of TB [13,14]. *In vitro* studies of IFN- γ , IL-4, TGF- β , and IL-1 β produced by peripheral blood mononuclear cells from tuberculosis patients [15], as well as reports wherein analysis

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of TNF alpha concentrations in BAL fluid [16] showed that the greater the amount of lung involvement the higher the cytokine production.

We report 24 patients with several TB clinical forms where sera measurements of TNF alpha had been made. We tried to associate serum TNF alpha levels with the evolution of clinical forms of TB. We concluded that serum TNF alpha measurement might play an important role in the evaluation of the inflammatory phenomena in TB.

Material and Methods

Sera were obtained from 24 patients, from the Infectious Diseases Clinic and Internal Medicine Clinic of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, with active TB and from 13 healthy individuals (control group). TB patients had a mean age of 32.95 years (18 to 72 years) with six females and eighteen males. The control group had a mean age of 34.84 years (21 to 47 years) with four males and nine females. Informed consent was obtained from each patient and the protocol for the research project was approved by the Ethics Committee of the Institution. We described 24 TB patients having the following clinical forms: 14 pulmonary, 2 pleural - pulmonary, 1 pleural - pulmonary + pericardial, 1 pleural + lymphatic, 1 pulmonary + lymphatic, 1 disseminated, 1 miliary, 2 peritoneal, 1 cutaneous + pulmonary. The TB diagnoses were established between 1993 and 2002.

Patients were all HIV negative and they were receiving anti-tuberculous therapy at the time of blood sampling. Medical records of all patients were analyzed for clinical data such as anemia, fever, weight loss, dyspnea, night sweats, cough, sputum, as well as diagnostic tests such as thoracic X-Ray (XR), computed tomographic (CT) scan of the chest, PPD skin test, erythrocyte sedimentation rate, and blood counts. The TB diagnosis was made if acid-fast bacilli had been positive in search or culture in each case (Table 2). We considered anemia as hemoglobin < 12 g/dL (woman) or < 14g/dL (man). Fever was defined as temperature $\geq 38^{\circ}\text{C}$. The clinical variables weight loss, dyspnea, night sweats, cough and sputum were registered as 0 (absent) or 1 (present). The results of the thoracic radiograph, the computed tomographic (CT) scan of the chest, and the sedimentation rate, were coded as 0 (normal) or 1 (altered). The PPD skin test was coded as 0 (negative) or 1 (positive). We also evaluated whether tissue destruction was detected by image diagnostic methods. The clinical severity of each case was determined through the following score: thoracic XR/CT normal=0; thoracic XR/CT altered without cavity=1; thoracic XR/CT altered with cavity=2; without anemia=0; with anemia=1; without hypoxia=0; with hypoxia=1; without weight loss=0; with weight loss=1.

TNF alpha Assay

TNF alpha bioactivity was determined by the McGee & Clemens bioassay method [17] with modifications, using L929 cells as targets. Briefly, L929 cells were cultured for 24 hours

on flat-bottom 96-well plates (1×10^4 cells/well), with RPMI 1640 medium at 37°C under an atmosphere of 5% CO_2 and 95% air. L929 cell monolayers were pre-incubated for 2 hours with 1 mg/mL of Actinomycin D (ActD). The patient's sera (stored at -80°C) were added to the wells and incubated for 18 hours. After this, the cells were stained with MTT vital stain. All samples were assayed at the same time. The readings were done using a spectrophotometer plate reader at a wavelength of 540 nm. The cell viability percentage was calculated using the formula: % cell viability=optical density (samples)/optical density (control) x 100. The final results of each sample were obtained through the average of eight wells. The serum TNF alpha level was calculated through a standard curve (recombinant TNF alpha *versus* cell viability percentage). The confidence interval over 95% of the control group (UCI 95%) was considered as the cut-off between individuals in the controls and individuals with TB.

Statistical Analysis

The data were separated into categorical and continuous variables. The categorical values were expressed in absolute frequency (n) and relative frequency (%), using Pearson Chi-square test. The continuous and semi continuous variables were compared to a normal curve, and classified as parametric or not using the K-S test. TNF alpha values were categorized using the superior 95% confidence interval. The parametric data were shown as mean \pm standard error, and compared using the unpaired Student's t-test. The nonparametric data were shown as median and 25th – 75th percentiles, and compared using the Mann-Whitney test. The ROC curve was used as the operational response curve to establish whether TNF alpha had good discriminating power. The correlations between the TNF alpha level in each case and all clinical and diagnostic variables were evaluated through the r-value calculation using Pearson's correlation. The alpha risk for all conditions was $\leq 5\%$ in type I error, and $\leq 20\%$ for beta risk in type II error.

Results

Demographic and Clinical Characteristics

The demographic data and TNF alpha levels of the 13 subject control group are shown in Table 1. The patients' general characteristics, TB clinical forms, length of symptoms and serum TNF alpha levels are shown in the Table 2. The days of therapy, clinical complications, length of treatment and clinical evolution of the patients with the TNF alpha levels are shown in Table 3. The mean of serum TNF alpha levels in patients with active TB was 13035.54 ± 15948.35 pg/mL, which was significantly higher than those found in healthy control individuals (341.3 ± 127.1 pg/mL) ($p < 0.01$).

Aspects of Severity and Evolution of Tuberculosis

The patients' distribution for clinical severity score is shown in Table 4. There was a positive correlation between the clinical severity score and the serum TNF alpha level ($r=0.44$; $p=0.045$) (Figure 1).

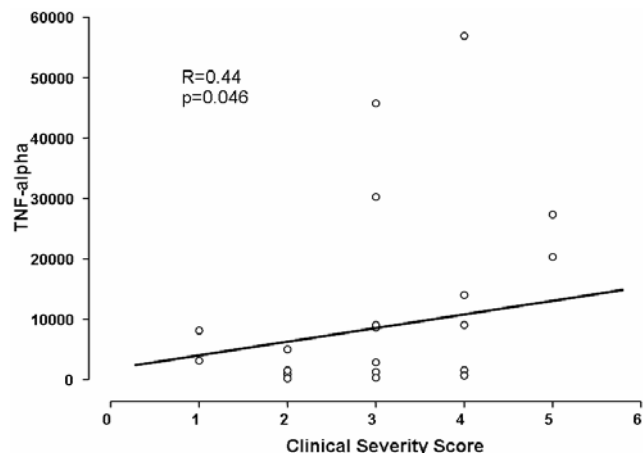
Table 1. Demographic characteristics of subject control group and TNF alpha levels.

Control	Age	Sex	TNF alpha (pg/mL)
1	27	F	1,415
2	35	F	0,49
3	21	M	8
4	40	M	45
5	40	F	314
6	48	F	598
7	41	F	389
8	50	F	314
9	37	F	133
10	32	F	15
11	35	F	45
12	31	M	1,141
13	53	M	19

Correlation Between Clinical or Laboratorial Variables and TNF Values

Table 5 shows statistical comparisons between clinical and laboratory variables of TB patients. There were no statistically significant differences between TNF alpha level and the following variables: anemia (p=0.148), fever (p=0.475), night sweats (p=0.859), dyspnea (p=0.514), cough (p=0.678) and sputum (p=0.182). Only the weight loss variable showed significant statistical difference in relation to TNF alpha. The patients with weight loss had higher serum TNF alpha (15468.54 \pm 4580.54 pg/mL) than those without weight loss (2904.98 \pm 1367.89 pg/mL) (p<0.05). The patients with abnormal chest X-rays had higher levels of TNF alpha (15328.48 \pm 4602.19 pg/mL) in relation to the patients with normal chest X-ray (3353.18 \pm 1495.29 pg/mL) (p<0.05). There was no difference between TNF alpha and sedimentation rate

Figure 1. Positive correlation between serum TNF-alpha levels and the clinical severity score.



(p=0.848), tissue destruction (p=0.149), multidrug-resistant TB (p=0.695) and clinical survival (p=0.352). The serum TNF alpha level was higher in patients with a positive PPD skin test when compared to those with a negative PPD test (p<0.05). TB treatment reduced significantly the serum TNF alpha values (p<0.05). A positive correlation was found between treatment duration and acid-fast bacilli (AFB) absence (r=0.717; p<0.05). In patients with successive TNF alpha measurements, a clinical improvement was always observed when serum TNF alpha levels dropped (patients 3, 7, 10, 14 and 19).

On the other hand, many patients without a decrease in serum TNF alpha levels presented clinical problems during their clinical evolution. For instance, patients 1 and 4 had pulmonary giant bubbles, patient 6 (MDR-TB) developed pulmonary cavitations, and patient 8 remained AFB positive in sputum until the fifth month of TB therapy.

The highest serum TNF alpha levels (> 15 x normal) were observed in 6 patients with severe TB: patient 1 (pulmonary TB with bubbles), patient 3 (pulmonary TB with cavitations), patient 4 (pulmonary TB with bubbles), patient 5 (pulmonary - MDR TB), patient 10 (pulmonary - interstitial TB) and patient 19 (disseminated TB). We found that high serum TNF alpha levels were associated with TB clinical forms having increased tissue destruction (shown by image methods). In three patients (15, 16 and 17), with pleural-pulmonary TB, high serum levels of TNF alpha were noted. In these patients the concomitant TNF alpha level in pleural fluid was undetectable.

The serum TNF alpha levels had good discriminant power in identifying patients with Tuberculosis (n=24) from control individuals (n=13). The control group upper confidence interval of 95% (UCI 95%) was 618.1 pg/mL. This value was used to categorize the continuous data. The ROC curve showed that the TNF alpha test was reliable (area=0.908 \pm 0.049, and p<0.0001).

Discussion

We studied 24 patients with various TB clinical forms. We tried to show a possible role of TNF alpha as a cytotoxic agent, mainly when high serum levels were observed (patients 1, 3, 5, 4, 10, 14 and 19). The presence of TNF alpha in the peripheral blood is a consequence of cytokine leakage to the serum. This cytokine is produced by tissues and inflammatory cells through monocyte-macrophage activation after *M. tuberculosis* stimulation [18].

The groups of patients presented illustrate the TNF alpha role in the various TB clinical forms. There was a marked difference of serum TNF alpha levels between the TB group and the control group. When the cases were classified by clinical severity score, we observed a positive correlation between clinical severity and serum TNF alpha levels. The subgroup analysis also showed interesting results. The MDR-TB cases (patients 5, 6, 7) presented severe pulmonary lesions with increased tissue destruction. In this group, the serum TNF alpha level ranged from a slight elevation (patients 6 and 7), to a very high level (patient 5).

Table 2. Demographic and clinical characteristics of patients with tuberculosis and TNF alpha levels.

Cases	Age	Sex	Clinical form	Symptoms length	TB diagnosis	TNF alpha*
1	40	M	Pulmonary	12 months	AFB \dagger S + C \ddagger in sputum	27319.0 \pm 4962.4 (4)
2	28	F	Pulmonary	2 weeks	AFB S + C in sputum	9055.0 (1)
3	50	M	Pulmonary	6 months	AFB S + C in BAL \S	14006.9 \pm 19431.4 (2)
4	18	M	Pulmonary	2 months	AFB S + C in sputum	20328.0 \pm 3920.2 (2)
5	20	F	Pulmonary	4 months	AFB S + C in sputum	56840.0 (1)
6	20	F	Pulmonary	2 months	AFB S + C in sputum	1551.4 \pm 450.5 (2)
7	31	F	Pulmonary	12 months	AFB S + C in sputum	719.5 \pm 615.9 (2)
8	27	M	Pulmonary	6 months	AFB S + C in sputum	1273.5 \pm 224.1 (2)
9	47	M	Pulmonary	12 months	AFB S + C in sputum	0 \ddagger (1)
10	61	M	Pulmonary	6 months	Lung biopsy	30221.0 \pm 22600.5 (2)
11	72	M	Pulmonary	2 months	AFB S + C in BAL	865.2 (1)
12	19	M	Pulmonary	4 months	AFB S + C in BAL	0 (1)
13	24	M	Pulmonary	2 months	AFB S + C in sputum	966.2 \pm 142.9 (2)
14	62	M	Pulmonary	2 weeks	AFB S + C in BAL	8620.0 \pm 5987.8 (2)
15	32	M	Pleural-pulmonary	2 months	AFB S + C in pleural fluid	9055.6 (1)
16	20	M	Pleural-pulmonary	1 month	AFB S + C in pleural biopsy	1519.9 (1)
17	19	M	Pleural-pulmonary + pericardial	5 months	AFB S + C in pleural biopsy	2848.0 (1)
18	21	M	Pleural + lymphatic	2 months	AFB S + C in lymph node biopsy	187.4 (1)
19	24	M	Disseminated \P	2 months	AFB S + C in lymph node biopsy	45703.0 \pm 5465.9 (2)
20	21	F	Lymphatic + pulmonary	3 months	AFB S + C in BAL and lymph node biopsy	303.9 (1)
21	32	F	Miliary	15 days	AFB C in sputum	0 (1)
22	50	M	Peritoneal	18 months	AFB C in ascitic fluid + peritoneal biopsy	3162.3 (1)
23	23	M	Peritoneal	4 months	Peritoneal biopsy	5000.0 (1)
24	30	M	Cutaneous + pulmonary	12 months	AFB S + C in sputum and skin abscess	8112.3 (1)

* TNF alpha values are in pg/mL; Normal TNF alpha=618.1 pg/mL; Number of dosages are in parenthesis; \dagger =acid-fast bacilli; \ddagger =search + culture; \S =bronchoalveolar lavage; \ddagger =TNF alpha undetectable; \P =pulmonary, pleural, lymphatic, splenic and meningeal tuberculosis.

On the other hand, in three patients the serum TNF alpha was undetectable: patient 9 (pulmonary with cavitations TB), patient 12 (pulmonary TB), and patient 21 (miliary TB). Patient 21 had a negative PPD skin test. We speculate that in these patients the immune response to the infectious agent could have been very weak. In another two patients, normal serum TNF alpha levels were detected: patient 18 (pleural + lymphatic TB) and patient 20 (lymphatic + pulmonary TB). These patients had normal serum TNF alpha values in spite of the presence of extensive lymphadenopathy with necrosis detected using image methods. We suppose that there can be a compartmentalization of the immune response in some patients with lymphatic TB. Patients 1, 3, 4, and 5 showed the highest serum TNF alpha levels and they also had increased pulmonary tissue destruction. We suggest that TNF alpha may have a direct role in this phenomenon.

We quantified TNF alpha successively in 10 patients and observed that, when serum TNF alpha levels decreased during clinical evolution (patients 3, 7, 10, 14 and 19), a clinical improvement was always noted. In contrast, when serum TNF alpha levels did not decrease during the evolution, a bad clinical course was noted. These results indicate that the serum TNF alpha level may be a good marker to predict the TB patient's clinical evolution. Ribeiro-Rodrigues et al. [19] showed that decreases in sputum IFN- γ , TNF alpha, and IL-8 closely parallel and even precede mycobacterial clearance, and they concluded that these cytokines may be markers of disease activity and inflammation in TB.

In this study we also found a positive correlation between serum TNF alpha levels and weight loss, abnormal chest X-Rays and positive PPD skin tests. The positive correlation between weight loss and serum TNF alpha levels was also observed in other studies. Bossola et al. [20] showed that

Table 3. Severity data and evolution aspects of patients with tuberculosis and levels of TNF-alpha.

Cases	TNF-alpha (pg/mL)	Days of therapy	Clinical complications	Therapeutic scheme	Final evolution
1	20000.0	90	Giant bubbles with pulmonary emphysema	HRZ/6 months	Recovery
	28480.0	120			
	30398.0	141			
	30398.0	183			
2	9055.0	21	Cavitation in the left lung	HRZ/8 days	Recovery
3	27747.0	30	A big cavitation in the right upper lobe	HRZ/6 months	Recovery
	266.8	60			
4	17556.0	15	Giant bubbles in the right lung/ chronic hypoxia	HRZ/6 months	Death
	23100.0	30			
5	56840.0	15	MDR - TB*, volumetric reduction of the left lung and bronchiectasis	SHRZ/9 months ECRZ/3 months	Death
6	1232.8	6	MDR - TB; Cavitations and bronchiectasis in both lungs	HRZ/2 months ESC+/6 months	Recovery
	1870.0	42			
7	1155.0	2	MDR - TB; Cavitations in both lungs	HRZ/3 months IHCCA/6 months	Recovery
	284.0	21			
8	1115.0	150	None	HRZ/6 months	Recovery
	1432.0	171			
9	1000	30	Volumetric lung reduction and cavitations in both lungs	HRZ/6 months	Recovery
10	46202.0	12	None	HRZ/6 months	Recovery
	14240.0	19			
11	865.2	4	None	HRZ/6 months	Recovery
12	0	2	None	HRZ/6 months	Recovery
13	865.2	15	Thrombocytopenia	HRZ/15 days EES/6 months	Recovery
	1067.3	19			
14	12854.0	34	None	HRZ/6 months	Recovery
	4386.0	61			
15	9055.6	1	Respiratory failure	HRZ/2 days	Death
16	1519.9	1	None	HRZ/6 months	Recovery
17	2848.0	45	Pericardial effusion	HRZ/6 months	Recovery
18	187.4	30	Simultaneous Paracoccidioido-mycosis	HRZ/6 months	Recovery
19	49568.0	4	Necrosis in several tissues	HRZ/6 months	Recovery
	41838.0	44			
20	303.9	5	None	HRZ/6 months	Recovery
21	0	1	AgHBs+, AgHBe+, Anti-VHC+	HRZ/6 months	Recovery
22	3162.3	38	Ascites	HRZ/6 months	Recovery
23	5000.0	1	Ascites	HRZ/6 months	Recovery
24	8112.3	1	Skin nodules in chest, neck, legs and head with spontaneous leakage	HRZ/4 days	Death

HRZ: H=isoniazid, R=rifampin and Z=pyrazinamide; *=multidrug-resistant tuberculosis; ESC: E=ethambutol, S=streptomycin and CIP=ciprofloxacin; HCCA: H=isoniazid, CFZ=clofazimine, CIP=ciprofloxacin and AKC=amikacin; EES: E=ethambutol, ETH=ethionamide and S=streptomycin; ESZ: E=ethambutol, S=streptomycin and Z=pyrazinamide; SHRZ: S=streptomycin, H=isoniazid, R=rifampin and Z=pyrazinamide; ECRZ: E=ethambutol, CIP=ciprofloxacin, ROX=roxythromicin and Z=pyrazinamide.

serum TNF alpha concentrations were significantly higher in patients with cancer and severe weight loss when compared with patients having cancer and low weight loss. Cakir et al. [21] speculated that serum TNF alpha levels and leptin might be responsible for the weight loss in pulmonary tuberculosis patients. Another study also correlated the chronic administration of TNF alpha with weight loss in rats [22].

We observed that the patients with abnormal chest X-rays had higher serum levels of TNF alpha. Tsao et al. showed that the bronchoalveolar lavage fluid levels of TNF alpha, IL-1 β and IL-6 were all significantly higher in patients with large cavities than in patients without cavities or with smaller cavities [23,24] These authors also showed that the relative excess of both TNF alpha and IL-1 β , associated with soluble

Table 4. Distribution of the Tuberculosis patients by clinical severity score and serum TNF-alpha levels.

Cases	Thoracic X-Ray/CT	Anemia	Hypoxia	Weight loss	Clinical severity score	TNF alpha*
1	2	1	1	1	5	27319.0 \pm 4962.4 (4)
4	2	1	1	1	5	20328.0 \pm 3920.2 (2)
3	2	1	1	1	5	14006.9 \pm 19431.4 (2)
5	2	1	1	1	5	56840.0 (1)
9	2	1	1	1	5	1000 (1)
2	2	1	0	1	4	9055.0 (1)
6	2	1	0	1	4	1551.4 \pm 450.5 (2)
7	2	1	0	1	4	719.5 \pm 615.9 (2)
14	1	1	1	1	4	8620.0 \pm 5987.8 (2)
20	1	1	1	1	4	303.9 (1)
8	1	1	0	1	3	1273.5 \pm 224.1 (2)
10	1	1	0	1	3	30221.0 \pm 22600.5 (2)
15	1	0	1	1	3	9055.6 (1)
17	1	1	0	1	3	2848.0 (1)
19	1	1	0	1	3	45703.0 \pm 5465.9 (2)
21	1	1	0	0	2	0 (1)
11	1	1	0	0	2	865.2 (1)
12	1	0	0	1	2	0 (1)
13	1	1	0	0	2	966.2 \pm 142.9 (2)
16	1	1	0	0	2	1519.9 (1)
18	1	0	0	1	2	187.4 (1)
23	0	1	0	1	2	5000.0 (1)
22	0	1	0	0	1	3162.3 (1)
24	1	0	0	0	1	8112.3 (1)

*=Values of TNF alpha in pg/mL; Number of measurements are inside parenthesis.

TNF alpha-receptor secretion imbalance, could have a correlation with the pulmonary cavities in patients having active TB [24]. However, there were few studies in medical literature about the correlation between serum TNF alpha levels and abnormal X-Rays, and further studies are necessary to understand this phenomenon. We also found that the serum TNF alpha level was elevated in patients with a positive PPD skin test. Chu et al. [25] observed that the number of cells staining for TNF alpha and IL-1 in the skin of six normal individuals during the PPD – induced reaction persisted at elevated levels [25]. The authors speculated that TNF alpha might play an important role in the development of delayed-type hypersensitivity in man. Therefore, we added the information that a high serum TNF alpha level occurs in patients with positive PPD skin tests, and this phenomenon suggests a possible correlation between this cytokine serum level and its tissue action.

We described three cases with pleural-pulmonary TB (15, 16 and 17) in which a high serum TNF alpha level was observed without a concomitant increase of TNF alpha in the pleural fluid. Vankayalapati et al. [26] reported that IL-18 and IFN gamma concentrations are higher in the pleural fluid of TB patients than in patients with other diseases. In another study, pleural fluid TNF alpha levels and pleural fluid/serum TNF alpha were higher in tuberculous effusions than in other

exudates, but their diagnostic value appears to be poorer than that of ADA [27]. Our result differs from these studies [26,27] and we did not find a clear explanation. We speculate that there was a barrier separating the pleural fluid and the host's immune response due to pleural fibrosis present in patient 16. We observed that in three cases, 1, 19 and 14, the serum TNF alpha level increased after the treatment of TB began. However, we can not state whether there was concomitant clinical worsening in these cases. Bekker et al. [13] observed worsening of clinical condition in 16 patients during the first 42 days of treatment, with two deaths among those having severe pulmonary TB. All patients had increased serum TNF alpha and lactic acid levels. These authors associated the worsening clinical condition of these patients with the increase of serum TNF alpha levels. Such evidences show the importance of estimating the serum cytokine level at the beginning of TB treatment.

The relation between TNF alpha and TB stands out due to the reactivation of latent TB infection with treatment with tumor necrosis factor (TNF) antagonists [28] in patients with several diseases such as: rheumatoid arthritis [29], juvenile arthritis, ankylosing spondylitis, Crohn's disease, psoriasis, glomerulonephritis, sarcoidosis and Behcet's disease. These diseases are characterized by a Th1 type immune response associated with excess generation of TNF-alpha [30].

Table 5. Statistical comparisons of clinical and laboratorial variables related to serum TNF-alpha levels in TB patients.

Variables	TNF alpha*	p
Patient with TB	13035.54 \pm 15948.35	p < 0.01
Control Group	341.3 \pm 127.1	
Weight loss 1 §	15468.54 \pm 4580.54	p < 0.05
Weight loss 0 †	2904.98 \pm 1367.89	
Thoracic X-Ray 1	15328.48 \pm 4602.19	p < 0.05
Thoracic X-Ray 0	3353.18 \pm 1495.29	
PPD 1	21710 \pm 17710	p < 0.05
PPD 0	101.3 \pm 101.3	
Anemia 1	13592.57 \pm 4228.01	p=0.148
Anemia 0	5785.10 \pm 2812.07	
Fever 1	10799.27 \pm 4498.97	p=0.475
Fever 0	16672.10 \pm 6521.49	
Night sweats 1	11225.22 \pm 7789.59	p=0.859
Night sweats 0	12868.47 \pm 4306.21	
Dyspnea 1	14557.54 \pm 5350.22	p=0.514
Dyspnea 0	9703.46 \pm 4964.60	
Cough 1	10711.0 \pm 4916.57	p=0.678
Cough 0	13801.26 \pm 5424.93	
Sputum 1	6713.95 \pm 3083.72	p=0.182
Sputum 0	14782.53 \pm 4932.87	
VHS 1	16223.23 \pm 5634.26	p=0.848
VHS 0	18511.45 \pm 9813.49	
Tecidual destruction 1	17535.96 \pm 5485.07	p=0.149
Tecidual destruction 0	6912.60 \pm 4439.29	
MR - TB 1	19742.60 \pm 18548.71	p=0.695
MR - TB 0	11266.32 \pm 3345.66	
Recovery	22890.98 \pm 11513.85	p=0.352
Death	10026.92 \pm 3611.45	

*=values of TNF alpha in pg/mL (mean \pm standard deviation); MR=Multi-resistant tuberculosis
 § 1=presence of variable; †0=absence of variable.

Nowadays there is much knowledge about TNF alpha influences in tissue injury. Inflammatory cells and cytokines cause death by apoptosis both in inflammatory and tissue cells (pneumocytes, fibroblasts, endothelial, etc.). The extrinsic pathway of apoptosis is activated by receptors belonging to the TNF alpha-receptor superfamily. However, Ashkenasi and Dixit [30] state that TNF alpha rarely starts cellular apoptosis, unless cellular protein synthesis is blocked. In contrast, Krammer [31] states that TNF alpha may cause cellular death by apoptosis, overcoming Rel A protein resistance [32]. Apoptotic cell death caused by TNF alpha may be completely or partially blocked by NF- κ B and its antiapoptotic proteins.

Ciaramella et al. [33] showed that MTB-induced apoptosis is inhibited by a caspase-1 inhibitor (YVAD) and is associated with the maturation of IL-1 β . In addition, IL-1 β and TNF alpha were produced massively in the course of infection, and both were inhibited by YVAD. Keane et al. [34] described the apoptosis of alveolar macrophages infected by *M. tuberculosis*. Watson et al. [35] found various pulmonary histopathologic alterations in different mice species with acute infection by *M. tuberculosis*. They suggested that apoptosis occurred during

granuloma formation after infection by *M. tuberculosis*, through a FAS/FAS ligand-independent pathway. Taken together, these studies correlate apoptotic cell death with TNF alpha, and they highlight the role of this cytokine in TB cases.

One of the objectives of our study was to show that increased serum TNF alpha was followed by severe clinical TB with high tissue destruction. On the other hand, we observed that a decrease in the serum cytokine level occurred in parallel with patient clinical improvement. We speculate that a reduction of high TNF alpha serum levels may decrease tissue destruction, particularly when the cytokine serum level remains elevated for a long time. TNF alpha periodic measurements through bioassay or Elisa methods could help to understand tuberculous outcome and it should be included in the routine TB clinical evaluation in the near future. However, in order to establish definitive conclusions more research should be done. Nowadays the treatment of TB is directed specifically to *M. tuberculosis* elimination. We suggest that TNF alpha immune modulation should be evaluated in *M. tuberculosis* therapy in future studies, especially in cases with very high TNF alpha serum levels.

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