The CD4+/CD8+ Ratio in Pulmonary Tuberculosis: Systematic and Meta-Analysis Article

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Abstract

Background: The ratio of CD4+/CD8+ has been used as a clinically index to evaluate patients' immunity. Numerous researchers have studied CD4+/CD8+ ratio in pulmonary tuberculosis (PTB) patients. However, the change of CD4+/CD8+ ratio remains controversial. We present a meta-analysis of 15 case-control studies to identify the change of CD4+/CD8+ ratio in PTB patients.

Methods: We assessed heterogeneity of effect estimates within each group using I^2 test. Subgroup analysis was performed to explore the potential source of heterogeneity. To investigate further the potential publication bias, we visually examined the funnel plots. For robustness of results, we performed sensitivity analysis by removing studies. Data entry and analyses were carried out with RevMan 5.2 (The Nordic Cochrane Centre).

Results: Twelve peripheral blood studies were categorized into two subgroups. Eight studies presented a significant decrease of CD4+/CD8+ ratio in PTB cases compared to healthy subjects (SMD: -0.45; 95% CI -0.65–-0.25; I^2 = 7%). Other four studies researched on the newly diagnosed patients presented a more seriously and significantly decrease (SMD: -2.17; 95% CI -2.61–-1.74; I^2 = 37%). The pooled analysis of bronchoalveolar lavage fluid (BALF) studies showed a significant increase of CD4+/CD8+ ratio using Flow Cytometry (FCM) (SMD: 4.75; 95% CI 3.44–6.05; I^2 =0%).

Conclusion: The present meta-analysis indicated that there was a synthetic evidence for the reduced CD4+/CD8+ ratio in peripheral blood of PTB patients, especially newly diagnosed cases. However, the CD4+/CD8+ ratio in BALF was increased using method of FCM.

Keywords: Pulmonary Tuberculosis, CD4+/CD8+ Ratio, Meta-analysis

Introduction

Globally there was an estimated 8.6 million new cases of tuberculosis (TB) in 2013 and 1.3 million deaths (1). Thus, TB is still a challenge as a global health problem. Cell mediated immunity is particularly important in defense against Mycobac-terium tuberculosis (MTB) infection and many types of T lymphocytes including αβ receptor T lymphocytes (CD4+ or CD8+), cytotoxic T lymphocytes and γδ receptor T lymphocytes play their

role in this regard (2). CD4+ T cells have an essential role in this response and are supported by other T-cell subsets such as CD8+ T cells, γδ T cells (3). The T cell lymphocyte subset profile of the peripheral blood, particularly the CD4+/CD8+ ratio, is treated as an indicator of personal immune competence to infection.

Although decreased CD4+ T cell counts and changes in CD8+ T cell counts with low

CD4+/CD8+ ratio are pivotal immune abnormalities in HIV patients, TB may be a cause of non-HIV associated CD4+ lymphopenia (4). The ratio of CD4+/CD8+ now has been used as a clinically conventional index to evaluate TB patients' immunity. Despite the pivotal importance of CD4+ and CD8+ lymphocytes in antimicrobial immunity and many researchers have studied changes of CD4+/CD8+ ratio in pulmonary tuberculosis (PTB) cases, most reports of human disease are based on findings in peripheral blood and Bronchoalveolar Lavage fluid (BALF), but they have obtained not exactly the same result.

Our objectives were to synthesize evidence from case-control studies to identify the change of CD4+/CD8+ ratio in PTB patients compared to healthy subjects. We presented a meta-analysis of 15 case-control studies, which provided data on CD4+/CD8+ ratio simultaneously from PTB cases and healthy subjects to answer the following question: What is the change of CD4+/CD8+ ratio in PTB cases compared to healthy subjects, higher or lower? How strong is the change? The data are combined statistically to provide a quantitative estimate of CD4+/CD8+ ratio between PTB cases and healthy controls.

Methods

Search Strategy and Study Selection

To identify eligible studies that address the value of CD4+/CD8+ ratio in tuberculosis patients, we performed a systematic literature search to identify relevant studies in an electronic database, personal reference collections of experts, reference lists of papers of interest, and published review articles. Studies published in English were searched for through PubMed and Web of Science databases. Search terms included "Tuberculosis", "TB", "PTB", and "CD4+/CD8+ ratio". Searches were limited to articles about human studies from 1990 to August 2014. We updated our searches on August 1, 2014. Inclusion criteria of the studies were met if they 1) provided or computed data on CD4+/CD8+ ratio with mean ± SD; 2) included a control group of healthy subjects without TB; 3) Patients without HIV and EPTB; 4) provide clear

information of TB confirmation and information of included patients; and 5) provide clear documentation of the operative techniques for quantifying lymphocyte. If data were duplicated in more than one study, the most relevant study was included in the present analysis.

Quality assessment

Two investigators (Y.Y. and J.Q.) developed the search strategy and one investigator (Y.Y.) conducted the primary systematic search for all studies meeting the predetermined inclusion criteria. The titles and abstracts of eligible studies identified by the primary search were screened. Study quality assessment and data extraction were conducted if a study met all inclusion criteria and no exclusion criteria. A second investigator (J.Q.) checked study eligibility, quality assessment, and data extraction, for validity and consistency. Full-text papers of the identified citations were reviewed by both the primary and secondary investigators in order to select the final studies. Any discrepancy was resolved by consensus, and if needed, by consultation with the third investigator (Y.P.D.).

Data Extraction

The following data were extracted from each study: 1) the first author, year of publication, and journal; 2) the country where the study was conducted; 3) study design; 4) study population, number of cases/controls; 5) CD4+/CD8+ ratios with mean±SD; 6) Method of quantifying lymphocyte; 7) Methodological details of confirmation of TB.

Statistical Analysis

Heterogeneity of effect estimates within each group of studies were assessed by χ^2 –based Q - tests and I^2 tests, where $I^2 (\%) > 50\%$ or $P < 0.10$ was considered significantly heterogeneous (5). For continuous data, if quantitative method is the same, we adopted the weighted mean difference (MD) as our analysis index. If they used different measuring instruments or units for the same variable or there was large difference among the mean value of numerical analysis, the standardized mean difference (SMD) was adopted for analysis. We

calculated 95% confidence interval (CI) of all analysis. The fixed-effects model was first fitted for all outcomes, and if the assumption of homogeneity of studies was violated (I^2 was more than 50%), the random effects model was fitted (6). Subgroup analysis was performed to explore the potential source of heterogeneity. To investigate the potential publication bias, we visually examined the funnel plots. For robustness of results, we performed sensitivity analysis by removing studies. Data entry and analyses were carried out with RevMan 5.2 (The Nordic Cochrane Centre).

Results

Description of Studies
Figure 1 provides the flow chart indicating the literature review process. Based on the inclusion criteria, 41 full articles were retrieved and 15 of these were included in final analysis. Some studies were excluded for the following reasons: 1) no provision of specific data on CD4+/CD8+ ratio (7-14); 2) cases of PTB was not representative, patients were divided into different groups, such as, cavitary and non-cavitary cases (15), poor general condition and good general condition cases

(4), MDRTB and NRTB cases (16); 3) there was no comparison group for healthy subjects (17-21); 4) there were EPTB and HIV patients included (22- 29); 5) the method of quantifying lymphocyte subpopulation was tentative (30, 31). All of these 15 studies were published in English. Table1 presents the characteristics of included 15 studies, n = 424 PTB cases and n = 307 healthy subjects reported. These included 10 studies researched on peripheral blood, 3 studies researched on BALF and 2 studies researched on peripheral blood and BALF simultaneously. Eleven studies quantified lymphocyte by FCM method and the rest by FM method. They were all case-control studies. Three studies (36.8%) were performed in the Iran (32-34), whereas two each (10.5%) were in China (35, 36) and Turkey (37, 38), one each (5.3%) in Netherland (39), Poland (40), Brazil (41), Kuwait (42), India (43), South Africa (44), Taiwan (45), and Saudi Arabia (46).

Outcome from eligible studies
Figure 2 summarizes the adjusted effect estimates of the peripheral blood studies, 12 studies are categorized into two subgroups.

Table 1: Characteristic of the included studies

Reference	Country	Design	Sample	Method	Control	Confirmation of TB
1 (31)	China	CC	PB	FCM	HC	Positive culture for M.tuberculosis.
2 (35)	Iran	CC	PB	FCM	HC	Sputum smear-positive and radiographs.
3 (32)	Iran	CC	PB	FCM	HC	Clinical, radiological and sputum smear examinations.
4 (33)	Netherland	CC	PB/BALF	FM	HC	Positive culture for M.tuberculosis, radiographs and histology.
5 (39)	Turkey	CC	PB	FCM	HC	Clinical, radiological and sputum smear examinations.
6 (37)	Poland	CC	PB	FCM	HC	Clinical, radiological examinations and sputum or bronchial washing culture.
7 (40)	Brazil	CC	PB	FCM	HC	Clinical, radiological signs and culture positive from fluid or pleural fragments.
8 (41)	Iran	CC	PB	FCM	HC	Sputum smear-positive and radiographs.
9 (34)	Kuwait	CC	PB	FCM	HC	Sputum-positive for TB.
10 (42)	India	CC	PB	FM	HC	Sputum-positive for acid-fast bacilli.
11 (43)	China	CC	BALF	FCM	HC	Sputum-positive for acid-fast bacilli and grew M.tuberculosis.
12 (36)	Turkey	CC	BALF	FM	HC	Clinical, radiological features and M.tuberculosis in sputum, aspirate, or SAL samples.
13 (38)	South Africa	CC	PB/BALF	FM	HC	Serum albumin, radiography, full blood counts and PPD.
14 (44)	Taiwan	CC	BALF	FCM	HC	Sputum smear-positive.
15 (45)	Saudi Arabia	CC	PB	FCM	HC	Symptoms of TB and sputum smear and/or culture positive.

CC case–control study, PB Peripheral blood sample, BALF Bronchoalveolar Lavage, FCM Flow Cytometry, FM Fluorescence Microscope, HC healthy controls.
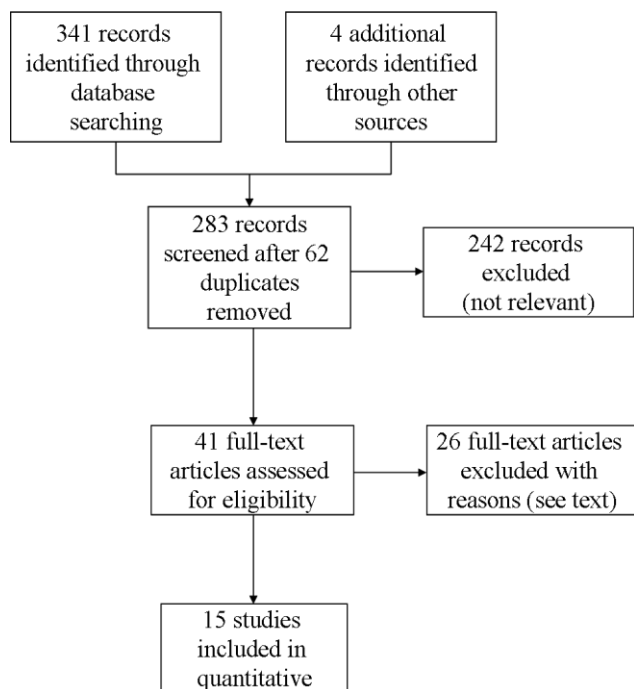
Fig. 1: Flow diagram of studies selection procedure

The forest plot shows a significant lower of CD4+/CD8+ ratio in pulmonary tuberculosis compared to healthy controls in the normal Peripheral blood studies (SMD: -0.45; 95% CI -0.65– -0.25; I^2 = 7%). There are 4 studies researched on the newly diagnosed and before treatment patients compared to healthy controls, which is different from other 8 studies, based on available data, CD4+/CD8+ ratio in these 4 studies decreased more seriously and significantly (SMD: -2.17; 95% CI -2.6 –-1.74; I^2 = 37%). Figure 3 summarizes the adjusted effect estimates of 5 BALF studies, which are categorized into two subgroups by the method of quantifying lymphocyte subpopulation, FCM (Flow Cytometry) and FM (Fluorescence Microscope). The forest plot shows a significant increase of CD4+/CD8+ ratio in pulmonary tuberculosis in studies of FCM (SMD: 4.75; 95% CI 3.44–6.05; I^2 =0%) , we did not report the summary effect estimates of the FM studies due to substantial heterogeneity (I^2: 93%).
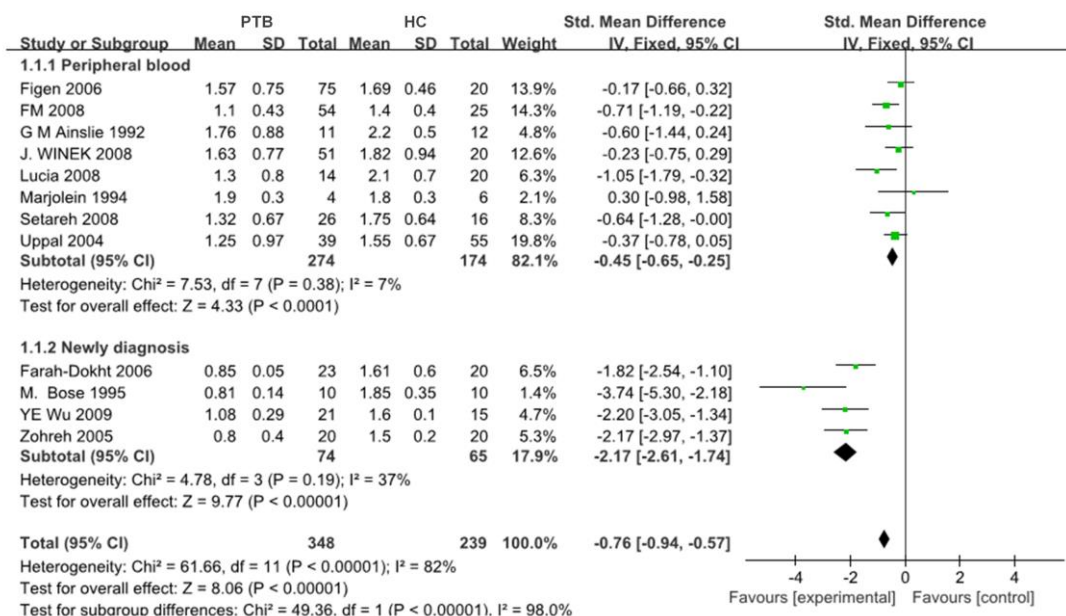
HC Healthy Control, PTB pulmonary tuberculosis.

Fig. 2: Forest plot of 12 peripheral blood studies on the CD4+/CD8+ ratio of PTB group and HC group
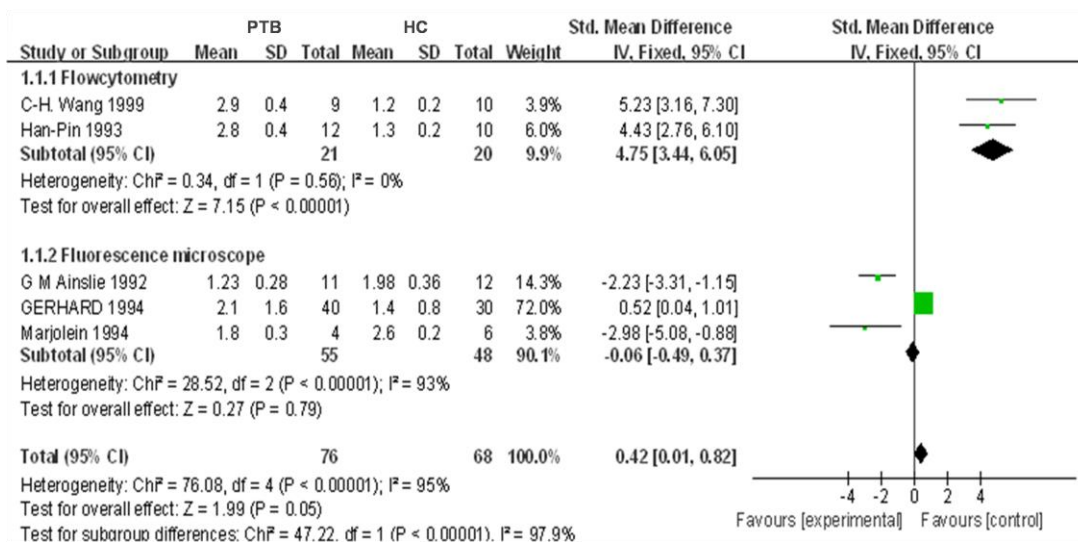
HC Healthy Control, PTB pulmonary tuberculosis.

Fig. 3: Forest plot of 5 BALF studies on the CD4+/CD8+ ratio of PTB group and HC group

Sensitivity Analysis

In the studies of peripheral blood, after removing a study with soldiers (42), the summary SMD was -0.47 (95% CI -0.71–-0.24; $I^2 = 18\%$. To analyze further the sensitivity of included studies, two studies were excluded because the method of quantifying lymphocyte subpopulation was Fluorescence Microscope instead of Flow Cytometry. The results of the sensitivity analysis show that with either Ainslie's or Marjolein's study excluded, the summary SMD were -0.46 (95% CI -0.71–-0.22; $I^2 = 31\%$) and -0.50 (95% CI -0.74–-0.26; $I^2 = 15\%$), respectively, were still near to the results before they were excluded. Regarding the subgroup of newly diagnosis, after removing a study which quantifying method was FM, the summary SMD was -0.95 (95% CI -2.49–-1.59; $I^2 = 0\%$), the result was also stable. With regard to BALF studies, we could not make the summary estimation in FM subgroup because of a substantial heterogeneity among FM studies (I^2: 93%) and there is no need to analyze the sensitivity in the FCM subgroup because of there are only two studies. Hence, the results of the sensitivity analysis indicate that the results of our study are reliable and believable.

Publication bias

We assessed the publication bias of the literature by the funnel plot in studies of peripheral blood and BALF, respectively. Funnel plot analysis did not detected obvious publication bias as the shape of the funnel plot did not reveal any evidence of obvious asymmetry (not shown).

Discussion

T-lymphocyte subsets, particularly CD4+ and CD8+ T cells, play a crucial role in immunity against mycobacterium infections (3, 47). In general, there were numerous researchers have studied changes of CD4+/CD8+ ratio (index of cell mediated immunity) in peripheral blood and BALF with TB patients, but they have obtained not exactly the same results.

Several studies indicated that there was a significant decrease of CD4+/CD8+ ratio in peripheral blood in pulmonary TB cases due to reduced CD4+ T cells counts, but the change of CD8+ T cells was inconsistent (41, 46). Others indicated that there was no significant change of CD4+/CD8+ ratio but significantly decreased in number of CD4+T cells (37, 40, 42), and the change of CD8+ T cells was reduced or not

changed. The studies researched on lymphocyte subpopulation in newly diagnosed TB cases before treatment showed that the CD4+/CD8+ ratio in peripheral blood was significantly lower in newly-diagnosed TB patients with a decrease of CD4+ T cell counts and an increase of CD8+ T cell counts (35, 32, 33). And, after several months of anti-tuberculosis therapy, the CD4+/CD8+ ratio in newly-diagnosed cases showed clinical improvement, and recorded a rise to near normal (33, 43).

However, the results of CD4+/CD8+ ratio in bronchoalveolar lavage (BALF) were different. Hanpin's result showed that the CD4/CD8 ratio was significantly higher in TB patients, when compared with corresponding controls (42). The similar result was reported by Wang's group (45). Gerhard's research also showed that the CD4/CD8 ratio was slightly higher in tuberculosis patients than in control subjects, but the difference was not statistically significant (38). Actually, the reduced CD4/CD8 ratio in BALF was also reported (39, 44).

These findings accord with the results obtained from previous studies that suggest a decreased CD4+ lymphocyte percentage in the peripheral blood of TB patients. This could be interpreted as indicating that CD4+ cells are critical for M tuberculosis infection. Regarding to the change of CD8+ lymphocyte percentage, and the role of CD8+ T cells in human immune responses to M tuberculosis, is not well defined. From these findings, we can hardly get a conclusion for the change of CD4+/CD8+ ratio in TB cases by consensus except in the newly diagnosed TB patients, four studies uniformly indicated the notable decreased CD4+/CD8+ ratio in the peripheral blood. We synthesized 12 studies to compare CD4+/CD8+ ratio in the peripheral blood from healthy controls and patients. Our results strongly indicated that CD4+/CD8+ ratio in peripheral blood reduced significantly, especially in the newly diagnosed subgroup. However, CD4+/CD8+ ratio in BALF studies was completely different with the result of peripheral blood studies. We used meta-analysis to synthesize 5 studies, which are categorized into two subgroups by the method

of quantifying lymphocyte subpopulation, FCM (Flow Cytometry) and FM (Fluorescence Microscope). The synthesized result showed a significant increase of CD4+/CD8+ ratio in pulmonary tuberculosis in studies of FCM. However, we did not report the summary effect estimates of the FM studies due to substantial heterogeneity.

Several limitations of our study should be considered. First, the studies retrieved in the analysis were full text in English searched on PubMed and Web of Science, so publication bias cannot be excluded. Second, the number of studies and participants for analysis was not large enough for analysis such as only four studies investigated in subgroup of newly diagnosis and only five studies researched on BALF. Furthermore, due to lack of appropriate data, the association of CD4+/CD8+ ratio and other important clinical parameters was not explored. Thus, better-designed studies are needed to present results that are more reliable.

Conclusion

The present meta-analysis indicates that there is synthetic evidence for the reduced CD4+/CD8+ ratio in peripheral blood in TB cases, especially newly diagnosed cases, the reduction is more severe. Differently, the CD4+/CD8+ ratio in BALF in TB cases is increased in the studies of using FCM method to quantify lymphocyte subpopulation. Further studies to understand the association between *M. tuberculosis* and the value of CD4+/CD8+ ratio in different parts of patients are recommended. Moreover, there is a need to further evaluate the mechanisms leading to these changes so as to understand the pathogenesis and prognostic markers of the disease and to develop immunomodulatory methods of therapy.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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References

1. World Health Organization (2013). Global tuberculosis report 2013. Geneva: WHO. Available from: http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_ eng. pdf.

2. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MP (2013). The immune response in tuberculosis. *Annu Rev Immunol*, 31:475-527.

3. Boom WH, Canaday DH, Fulton SA (2003). Human immunity to M. tuberculosis: T cell subsets and antigen processing. *Tuberculosis*, 83(1-3): 98-106.

4. Al-Aska A, Al-Anazi AR, Al-Subaei SS, Al-Hedaithy MA, Barry MA, Somily AM, Buba F, Yusuf U, Al Anazi NA (2011). CD4+ T-lymphopenia in HIV negative tuberculous patients at King Khalid University Hospital in Riyadh, Saudi Arabia. *Eur J Med Res*, 16(6):285-8.

5. Higgins JPT, Thompson SG, Deeks JJ (2003). Measuring inconsistency in meta-analyses. *Br Med J*, 327(7414): 557-60.

6. Higgins JPT, Thompson SG (2002). Quantifying heterogeneity in a meta-analysis. *StatMed*, 21(11): 1539-58.

7. Kony SJ, Hane AA, Larouzé B (2000). Tuberculosis-associated Severe CD4+T-Lymphocytopenia in HIV-Seronegative Patients from Dakar. *J Infect*, 41(2): 167-71.

8. Thomas CY Tsao, Chi-Hong Chen, Ji-hong Hong (2002). Shifts of T4/T8 T lymphocytes from BAL fluid and peripheral blood by clinical grade in patients with pulmonary tuberculosis. *Chest*, 122(4):1285-91.

9. Nadeem A, Khursheed J, Saleem-uz-Z (2010). Percentage of CD4+ and CD8+ T-lymphocytes in blood of tuberculosis patients. *J Ayub Med Coll Abbottabad*, 22(4): 182-6.

10. Adel Almogren (2011). Pulmonary tuberculosis associated with increased number and percentage of natural killer and B cells in the peripheral blood. *Afr J Microbiol Res*, 5(15): 2048-52.

11. Rodrigues DS, Medeiros EA, Weckx LY (2002). Immunophenotypic characterization of peripheral T lymphocytes in mycobacterium tuberculosis infection and disease. *Clin Exp Immunol*, 128(1):149-54.

12. Gambon-Deza F, Pacheco Carracedo M, Cerda Mota T, Montes Santiago J (1995). Lymphocyte populations during tuberculosis infection: Vβ repertoires. *Infect Immun,* 63(4): 1235-1240.

13. Balikoè Z. Szereday L. Szekeres-Bartho J (1998). Th2 biased immune response in cases with active Mycobacterium tuberculosis infection and tuberculin anergy. *FEMS Immunol Med Microbiol*, 22(3): 199-204.

14. Uh ST, Ki SY, Lim GI, Moon SH, Jeong SW, Kim HT, Kim YH, Park CS (1998). The T cell receptor subsets of lymphocytes in bronchoalveolar lavage in patients with active pulmonary tuberculosis. *Respir Med*, 92(3): 408-14.

15. Mazzarella G, Bianco A, Perna F, D'Auria D, Grella E, Moscariello E, Sanduzzi A (2003). T lymphocyte phenotypic profile in lung segments affected by cavitary and non-cavitary tuberculosis. *Clin Exp Immunol*, 132(2): 283-8.

16. Yildiz P, Kadakal F, Tütüncü Y, Deniz G, Gürel N, Adin S, Yilmaz V (2001). Natural Killer Cell Activity in Multidrug-Resistant Pulmonary Tuberculosis. *Respiration*, 68(6): 590-4.

17. Jafari C1, Ernst M, Strassburg A, Greinert U, Kalsdorf B, Kirsten D, Lange C (2008). Local immunodiagnosis of pulmonary tuberculosis by enzyme-linked immunospot. *Eur Respir J*, 31(2): 261-5.

18. Mirsaeidi M, Shah Ghasempour S, Valiollahpour Amiri M (2002). Lymphocyte sub-populations and lymphocyte activation markers in pulmonary TB patients. *Tanaffos*, 1(4): 37-44.

19. Faith A, Schellenberg DM, Rees AD, Mitchell DM (1992). Antigenic specificity and subset analysis of T cells isolated from the bronchoalveolar lavage and pleural effusion of patients with lung diseases. *Clin Exp Immunol*, 87(2): 272-8.

20. Turett GS, Telzak EE (1994). Normalization of

CD4+ T-lymphocyte depletion in patients without HIV infection treated for tuberculosis. *Chest*, 105(5): 1335-7.

21. Yu CT, Wang CH, Huang TJ, Lin HC, Kuo HP (1995). Relation of bronchoalveolar lavage T lymphocyte subpopulations to rate of regression of active pulmonary tuberculosis. *Thorax*, 50(8): 869-74.

22. Sehgal VN, Wagh SA, Bhattacharya SN, Sharma VK (1992). Peripheral T lymphocytes and their subsets in cutaneous tuberculosis. Int J Dermatol, 31(2): 110-2.

23. Hong SY, Wang XL (1991). Clinical Effects and Immune Regulation of Musk-Moxa-String Therapy in 39 Patients with Scrofula. *Zhong Xi Yi Jie He Za Zhi*, 11(8): 465-7.

24. Qama D, Choi WI, Kwon KY (2012). Immune responses in the lungs of patients with tuberculous pleural effusion without pulmonary tuberculosis. *BMC Immunol*, 13(13):45.

25. Caramori G, Lasagna L, Casalini AG, Adcock IM, Casolari P, Contoli M, Tafuro F, Padovani A, Chung KF, Barnes PJ, Papi A, Rindi G, Bertorelli G (2011). Immune Response to Mycobacterium tuberculosis Infection in the Parietal Pleura of Patients with Tuberculous Pleurisy. *PLoS ONE*, 6(7): e22637.

26. Dlugovitzky D1, Rateni L, Torres-Morales A, Ruiz-Silva J, Piñesky R, Canosa B, Molteni O, Bottasso O (1997). Levels of interleukin-8 in tuberculous pleurisy and the profile of immunocompetent cells in pleural and peripheral compartments. *Immunol Lett*, 55(1): 35-39.

27. Jones BE, Oo MM, Taikwel EK, Qian D, Kumar A, Maslow ER, Barnes PF (1997). CD4 cell counts in human immunodeficiency virus-negative patients with tuberculosis. *Clin Infect Dis*, 24(5): 988-91.

28. Albera C, Mabritto I, Ghio P, Scagliotti GV, Pozzi E (1991). Lymphocytes subpopulations analysis in pleural fluid and peripheral blood in patients with lymphocytic pleural effusions. *Respiration*, 58(2): 65-71.

29. Nikolaeva LG, Maystat TV, Pylypchuk VS, Volyanskii YL, Masyuk LA, Kutsyna GA (2008). Effect of oral immunomodulator Dzherelo in TB/HIV co-infected patients receiving anti-tuberculosis therapy under DOTS. *Int Immunopharmacol*, 8(6): 845-51.

30. Liu C, Teng Q, Xing S (1996). 0soluble interleukin-2 receptor, expression of membrane inter-leukin-2 receptor and T lymphocyte subpopulations in pulmonary tuberculosis. *Chin J Tuberc Respir Dis*, 19(1): 44-6.

31. Fan XH, Gao H (1990). Determination of lymphocyte subsets in pulmonary tuberculosis by Mc-Ab- rosette reagent method. *Zhonghua Jie He He Hu Xi Za Zhi*, 13(5): 297-298.

32. Fateminasab FD, Shahgasempour S, Mirsaeidi SM, Tabarsi P, Mansoori SD, Entezami Z (2006). Increased Activation And Expression Of A CD57+ Subset Within Peripheral CD8+ T Lymphocytes In Mycobacterium Tuberculosis-infected Patients. *Arch Iranian Med*, 9(1): 53-57.

33. Pessaran Z, Sahebfosul F, Oreizi F, Ghavaminejad A, Kiani A, Dana Siadat Z (2005). Immuno-phenotypic Characterization of Peripheral Blood T-Lymphocytes and Their Subpopulations in Tuberculosis Patients before and after Treatments. *Iran J Allergy Asthma Immunol*, 4(1): 23-6.

34. Davoudi S, Rasoolinegad M, Younesian M, Hajiabdolbaghi M, Soudbakhsh A, Jafari S, EmadiKouchak H, Mehrpouya M, Lotfi H (2008). CD4+ Cell Counts in Patients with Different Clinical Manifestations of Tuberculosis. *Braz J Infect Dis*, 12(6): 483-6.

35. Wu YE, Zhang SW, Peng WG, Li KS, Li K, Jiang JK, Lin JH, Cai YM (2009). Changes in Lymphocyte Subsets in the Peripheral Blood of Patients with Active Pulmonary Tuberculosis. *J Int Med Res*, 37(6): 1742-9.

36. Kuo HP, Yu CT (1993). Alveolar macrophage subpopulations in patients with active pulmonary tuberculosis. *Chest*, 104(6):1773-8.

37. Deveci F, Akbulut HH, Celik I, Muz MH, Ilhan F (2006). Lymphocyte Subpopulations in Pulmonary Tuberculosis Patients. *Mediators Inflamm*, 2006(2):89070.

38. Hoheisel GB, Tabak L, Teschler H, Erkan F, Kroegel C, Costabel U (1994). Bronchoalveolar lavage cytology and immunocytology in pulmonary tuberculosis. *Am J Respir Crit Care Med*, 149(2 Pt 1): 460-3.

39. Drent M, Wagenaar SS, Mulder PH, van Velzen-Blad H, Diamant M, van den Bosch JM (1994). Bronchoalveolar Lavage Fluid Profiles in Sarcoidosis, Tuberculosis, and Non-Hodgkin's and Hodgkin's Disease. *Chest*, 105(2): 514-9.

40. Winek J, Rowinska-Zakrzewska E, Demkow U, Szopinski J, Szolkowska M, Filewska M, Jagodzinski J, Roszkowski-Sliz K (2008). Inter-

feron Gamma Production In The Course Of Mycobacterium Tuberculosis Infection. *J Physiol Pharmacol*, 59 Suppl 6: 751-9.

41. Aguiar LM, Antonangelo L, Vargas FS, Zerbini MC, Sales MM, Uip DE, Saldiva PH (2008). Malignant and Tuberculous Pleural Effusions: Immunophenotypic Cellular Characterization. *Clinics*, 63(5): 637-44.

42. Uppal SS, Tewari SC, Verma S, Dhot PS (2004). Comparison of CD4 and CD8 Lymphocyte Counts in HIV-Negative Pulmonary TB Patients With Those in Normal Blood Donors and the Effect of Antitubercular Treatment: Hospital-Based Flow Cytometric Study. *Cytometry B Clin Cytom*, 61(1): 20-6.

43. Bose M, Gupta A, Banavalikart JN, Saha K(1995). Dysregulation of homeostasis of blood T-lymphocyte subpopulations persists in chronic multibacillary pulmonary tuberculosis patients refractory to treatment. *Tuber Lung Dis*, 76(1):

59-64.

44. Ainslie GM, Solomon JA, Bateman ED (1992). Lymphocyte and lymphocyte subset numbers in blood and in bronchoalveolar lavage and pleural fluid in various forms of human pulmonary tuberculosis at presentation and during recovery. *Thorax*, 47(7): 513-8.

45. Wang CH, Yu CT, Lin HC, Liu CY, Kuo HP (1999). Hypodense alveolar macrophages In patients with diabetes mellitus and active pulmonary tuberculosis. *Tuber Lung Dis*, 79(4): 235-42.

46. Majid FA, Abba AA (2008). Immunophenotypic characterization of peripheral T lymphocytes in pulmonary tuberculosis. *J Postgrad Med*, 54(1): 7-11.

47. Orme IM, Andersen P, Boom WH (1993). T cell response to Mycobacterium tuberculosis. *J Infect Dis*, 167(6): 1481-97.