Diagnostic Potential of Circulating Biomarkers in Adenosine Deaminase Diagnosed Pleural Tuberculosis Cases

Bineeta Kashyap^{1,2} · Nisha Goyal¹ · N. P. Singh¹ · Iqbal R. Kaur¹

Received: 17 May 2017 / Accepted: 21 July 2017 / Published online: 1 August 2017
© Association of Clinical Biochemists of India 2017

Abstract Pleural tuberculosis accounts for nearly 20% of Extra pulmonary tuberculosis. Adenosine deaminase, commonly used biomarker for the diagnosis, is non specific and there is paucity of literature on its correlation with conventional or newer methods for the diagnosis of extra pulmonary forms of TB. The aim of the study was to assess diagnostic potential of T cell function markers [interferon (IFN- γ), interleukin (IL-2) and IFN- γ /IL-2 ratio]; macrophage activation marker [neopterin]; and oxidative stress markers [protein carbonyl and malondialdehyde (MDA)] in pleural tuberculosis. 26 pleural TB cases diagnosed on the basis of suggestive chest X-ray and raised serum ADA levels and healthy controls were included in the study. Pleural fluid specimens were subjected to Zeihl Neelsen staining and culture on Lowenstein Jensen medium. Serum IFN- γ , IL-2, neopterin and protein carbonyl levels detection were done by ELISA and MDA levels were determined by measuring the thiobarbituric acid reactive substances. Median serum levels of IFN- γ , IL-2, IFN- γ /IL-2 ratio, neopterin, protein carbonyl and MDA were significantly different between cases and controls. Levels of all biomarkers except IL-2 were significantly higher in cases with contact history. Mean levels of ADA and ESR were 46.27 U/L and 46.62 mm/hr in PTB cases. AUC for IFN- γ , IL-2, IFN- γ /IL-2 ratio, neopterin, protein carbonyl and MDA were significantly discriminative for cases and controls. IFN- γ /IL-2 ratio was best discriminatory biomarker with highest area under ROC curve.

Though no correlation was seen between ADA and any of the six biomarkers, ESR levels correlated significantly with all biomarkers except IL-2 by spearman's correlation coefficient. Though all the circulating biomarkers under study provide useful supportive evidence for the diagnosis of PTB, further studies involving diverse control groups particularly non-PTB effusion are needed to validate these results.

Keywords Pleural · Tuberculosis · ADA · Biomarkers

Introduction

As per WHO Global TB Report 2015 the epidemic of tuberculosis (TB) is more extensive than previously perceived. India, one of the six countries constituting 60% of the global burden of new cases, accounted for the largest number of cases worldwide. In the global 9.6 million incident TB cases annually, India alone contributed 2.2 million new TB cases [1]. TB is caused by *Mycobacterium tuberculosis* and can potentially involve any part of the body. Though among the total TB load, pulmonary tuberculosis is the most prevalent form of TB, extrapulmonary cases account for nearly 15–20% in immunocompetent and 50% in HIV co-infection cases [2]. In extrapulmonary tuberculosis (EPTB) any organ other than the lungs parenchyma is involved. Pleural tuberculosis accounts for nearly 20% of EPTB cases [3]. Pleural tuberculosis is frequently associated with primary TB disease and can occur due to the contiguous spread from lung parenchymal inflammation or as a result of post-primary TB disease.

Very often the detection of EPTB cases is not straightforward. In a large proportion of EPTB cases, clinical signs and symptoms are non-specific, hence, the tests for the diagnosis of EPTB are performed late in the course of

✉ Bineeta Kashyap
dr_bineetakashyap@yahoo.co.in

¹ Department of Microbiology, University College of Medical Sciences & Guru Teg Bahadur Hospital, New Delhi, India

² Flat no. C-402, Vimal CGHS LTD., Plot-3, Sector-12, Dwarka, New Delhi 110078, India

disease; even after the failure of empirical antibiotic therapy [4]. Moreover, the sensitivity of conventional diagnostic methods of direct microscopy for acid fast bacilli (AFB) and culture on solid media is relatively low due to the pauci-bacillary nature of extra pulmonary specimens. Another major constraint is the long mean incubation period of 4–6 weeks for the growth of *Mycobacterium* on cultivation as it has a slow rate of growth. In some cases the diagnosis of pleural tuberculosis is made on the basis of histopathology findings in a pleural biopsy specimen. The invasive sampling done in these cases cannot be repeated easily, besides requiring technical expertise.

Thus, to address these problems, efforts are now being directed towards detection of suitable biomarkers for the diagnosis of pleural tuberculosis. Biomarkers can be defined as the measurable characteristics that indicate normal biological or pathogenic processes or pharmacological responses to a therapeutic intervention. In order to prove useful as a surrogate marker of disease, a biomarker should have a direct role in disease pathogenesis or protection and must reflect the improvement in the pathology following suitable treatment [5]. In many settings estimation of adenosine deaminase (ADA) levels is the most commonly used method for the diagnosis of pleural tuberculosis and further to start empirical treatment. Adenosine deaminase is the enzyme involved in purine catabolism and also has a role in the proliferation and differentiation of lymphocytes [6]. Elevated ADA levels are not specific for the diagnosis of pleural tuberculosis as levels can also increase above the baseline values in several other pathologies [7]. Moreover, there is a paucity of literature on the correlation of ADA with the other conventional or newer methods for the diagnosis of extra pulmonary forms of TB. Erythrocyte sedimentation rate (ESR) is another non-specific marker of inflammation often assessed in association with TB. The present study was initiated to assess the diagnostic utility of various biomarkers in pleural tuberculosis cases diagnosed on the basis of ESR, chest X-ray findings and ADA levels in pleural fluid. The aim of the study was to estimate the diagnostic potential of circulating biomarkers directly involved in the pathogenesis of TB including T-cell function markers [interferon (IFN- γ), interleukin (IL-2) and IFN- γ /IL-2 ratio]; macrophage activation marker [neopterin]; and oxidative stress markers [protein carbonyl and malondialdehyde (MDA)] in pleural tuberculosis cases.

Materials and Methods

A cross-sectional study was carried out in the Immunology section, Department of Microbiology, of a tertiary care hospital over a period of one year. This study included 26

pleural tuberculosis cases diagnosed on the basis of ESR, suggestive chest X-ray findings and raised serum ADA levels with 26 age and sex matched healthy controls. Contact history was obtained in all the cases. To rule out latent TB, only the healthy subjects with a negative tuberculin skin test were included in the control group. Any pleural tuberculosis patient with a coexisting systemic disease, immunodeficiency, malignancy or autoimmune disorder were excluded from our study. The Institutional Ethical Committee gave approval for this study. A written informed consent was taken from all the study subjects prior to being included in the study.

Sera were separated from the blood specimens of both the cases and healthy controls and the aliquoted sera were stored at -80°C till further processing. In addition, a pleural fluid specimen was obtained from all the cases of pleural tuberculosis included in our study. All pleural fluid specimens were subjected to direct microscopy for the demonstration of acid fast bacilli by Ziehl–Neelsen (ZN) staining and were cultured on Lowenstein–Jensen (LJ) medium for *M. tuberculosis* isolation.

Detection of Serum IFN- γ , IL-2, neopterin and protein carbonyl was done by using the commercially available enzyme linked immunosorbent assay (ELISA) kits following the manufacturers' instructions. Diaclone Human ELISA kit (France) was used for determining the serum IFN- γ and IL-2 levels which had a sensitivity of <5 and 7 pg/ml , respectively. The principle of solid-phase sandwich ELISA was the basis of both these assays. DRG, DRG Instruments GmbH, Human Neopterin ELISA kit (Germany) was used for the estimation of serum neopterin levels. It used the principle of a solid phase competitive ELISA. Protein carbonyl levels was assessed by QAYBEE-BIO For Life Science Human Protein carbonyl (PCO) ELISA kit (China), with an assay range of $15.6\text{--}1000\text{ pg/ml}$ and it was based on the principle of double-antibody sandwich ELISA. The correlation of the absorbance of reference controls and the samples was used for the interpretation of the results. The levels of MDA were determined in by the estimation of the thiobarbituric acid reactive substances spectrophotometrically at 532 nm . MDA reacts with thiobarbituric acid and forms a pinkish-red coloured chromogen which has a maximum absorbance at 532 nm .

Statistical Analysis

Mann–Whitney U test was applied for the comparative analysis of the various biomarkers under study between the pleural tuberculosis cases and healthy controls and the p values were calculated for the assessment of difference in distribution of biomarkers between these two groups. Mann–Whitney U test and p values were also used for the

assessment of circulating biomarkers under study among cases with and without a history of contact with known tuberculosis case. For the analysis of diagnostic efficacy of various biomarkers under study in pleural tuberculosis cases, receiver operating characteristics (ROC) curve analysis was used. Spearman's correlation coefficient (ρ) was used to assess the correlation among circulating biomarkers with ESR levels and pleural fluid ADA levels in cases group.

Results

In this study the age of patients ranged from 18 to 62 years. Majority of the pleural tuberculosis patients belonged to 18–30 years followed by 31–45 years. A male to female ratio of 1.9 was seen depicting a male preponderance (Fig. 1).

In 3 (11.5%) pleural fluid specimens acid fast bacilli was seen on direct microscopy and in 4 (15.4%) specimens *Mycobacterium tuberculosis* was isolated on culture. In 1 (3.8%) of the all pleural fluid specimens, both the direct

Fig. 1 Age and sex distribution of the pleural tuberculosis cases (n = 26)

microscopy for acid fast bacilli and isolation of *Mycobacterium tuberculosis* on culture was positive. The mean levels of ADA and ESR were 46.27 U/L and 46.62 mm/h in pleural tuberculosis cases.

Assessment of Circulatory Biomarkers in Pleural Tuberculosis Cases and Healthy Controls

Table 1 depicts the comparison of median serum levels of various biomarkers under study in between the pleural tuberculosis cases and healthy controls. The distribution of median serum levels of the biomarkers under study was found to be significantly different between the cases and the healthy controls ($p < 0.05$). The median serum levels of IFN- γ , IFN- γ /IL-2 ratio, neopterin, protein carbonyl and MDA were significantly higher in the pleural tuberculosis cases than the healthy controls. However, the median serum level of IL-2 was significantly higher in the healthy controls than the cases group.

The area under the curve (AUC) of all the biomarkers under study was able to significantly discriminate between the pleural tuberculosis cases and the healthy controls ($p < 0.05$). A separate receiver operating characteristics (ROC) curve for IL-2 has been made as the lower values of IL-2 favoured the cases whereas for all the other biomarkers under study higher values represented the cases (Table 1; Fig. 2).

Correlation Among Various Biomarkers Under Study with ESR and Pleural Fluid ADA in Pleural Tuberculosis Cases

A positive correlation was seen among the cases between ESR levels and the serum levels of all the biomarkers under study (except IL-2) by spearman's correlation. However, no such correlation was seen between the levels of any of

Table 1 Comparison of median serum levels and analysis of the receiver operating characteristics (ROC) curve for the diagnostic potential of circulating biomarkers among the pleural TB cases

Parameter	Biomarker under study					
	IFN- γ	IL-2	IFN- γ /IL-2	Neopterin	Malondialdehyde	Protein carbonyl
Median serum levels among cases (n = 26)	29.8 (10–226.9) (pg/ml)	8.1 (3.6–12.6) (pg/ml)	3.8 (2.2–56.1)	25.3 (5–111) (nmol/L)	7.5 (3.1–18.8) (nmol/ml)	684.1 (345.8–1502.8) (pg/ml)
Median serum levels among controls (n = 26)	5.0 (0.8–13) (pg/ml)	9.5 (3.8–18) (pg/ml)	0.6 (0.0–1.5)	4.8 (2–17) (nmol/L)	1.8 (1.1–5.8) (nmol/L)	349.2 (208–526.8) (pg/ml)
p value for median serum levels among cases and controls	<0.001	0.039	<0.001	<0.001	<0.001	<0.001
Area under the curve (AUC)	0.994	0.666	1.000	0.965	0.987	0.933
p value for AUC	<0.001	0.040	<0.001	<0.001	<0.001	<0.001
Standard error	0.006	0.075	0.000	0.022	0.014	0.032
95% CI	0.982–1.000	0.519–0.814	1.000–1.000	0.922–1.000	0.959–1.000	0.871–0.996

Fig. 2 Receiver operating characteristics (ROC) curve for the diagnostic efficacy of circulating biomarkers among the ADA diagnosed pleural TB cases

Table 2 Correlation of various circulating biomarkers under study with ESR and pleural fluid ADA levels in the study group (n = 26)

		ESR level (mm/hr)	Pleural fluid ADA levels (U/L)
IFN- γ	Spearman's rho	0.707	0.267
	p value	<0.001	0.188
IL-2	Spearman's rho	0.268	0.334
	p value	0.185	0.095
IFN- γ /IL-2 ratio	Spearman's rho	0.682	0.200
	p value	<0.001	0.326
Neopterin	Spearman's rho	0.611	−0.038
	p value	0.001	0.854
Malondialdehyde (MDA)	Spearman's rho	0.433	−0.051
	p value	0.027	0.804
Protein carbonyl	Spearman's rho	0.571	−0.011
	p value	0.002	0.958

the biomarkers and pleural fluid ADA levels. Table 2 shows spearman's correlation of various biomarkers under study with ESR levels and pleural fluid ADA levels in pleural tuberculosis cases.

Assessment of Circulatory Biomarkers in Pleural Tuberculosis Cases on the Basis of History of Contact

In pleural tuberculosis cases, 7(26.9%) reported a history of contact with a known case of tuberculosis. Table 3 demonstrates the comparison of the median serum levels of various

biomarkers under study among the pleural tuberculosis cases with and without a history of contact. Though the interpretation of median serum levels did not have a conclusive interpretation, a significant difference was seen in the median serum levels of all the biomarkers under study except IL-2.

Discussion

Although Tuberculosis affects primarily the lungs, extra-pulmonary TB may be the initial presentation in approximately 25% of adults that primarily affects the lymph

Table 3 Distribution of median serum levels of biomarkers under study in pleural TB cases with and without history of contact

Biomarker under study	Median serum levels among cases with contact history (n = 7)	Median serum levels among cases without contact history (n = 19)	p value
IFN- γ (pg/ml)	24.2	34.3	<0.001
IL-2 (pg/ml)	8.7	7.5	0.083
IFN- γ /IL-2	2.7	4.0	<0.001
Neopterin (nmol/L)	23.2	27.0	<0.001
Malondialdehyde (nmol/ml)	7.4	7.5	<0.001
Protein carbonyl assay (pg/ml)	706.9	661.3	<0.001

nodes and pleura [8]. Pleural tuberculosis is a diagnostic dilemma due to the limitations of traditional diagnostic tools used in extra pulmonary forms of tuberculosis [9]. The pathogenesis of tubercular pleural effusion is the manifestation of paucibacillary mycobacterial infection into the pleural space and thus the interaction with T cells after the rupture of subpleural caseous focus from the lung into the pleural space [10]. This interaction with T cells that are previously sensitized to Mycobacterium lead to a delayed hypersensitivity reaction [11]. The initial neutrophilic inflammatory response is followed by a predominantly lymphocytic reaction leading to the formation of a pleural granuloma, which probably explains the reduced likelihood of culture positivity as the viable mycobacteria are contained [12].

The definitive diagnosis of pleural TB can be done by demonstration of tuberculous bacilli either by microscopy and/or culture or demonstration of tuberculous granulomas with acid fast bacilli in the pleura with supportive evidence of elevated ADA levels in the pleural fluid [7, 9]. In endemic settings, however, the diagnosis is most often inferred in patients with high levels of ADA and an exudates with lymphocytic predominance. Though pleural fluid, pleural biopsy and sputum ideally should be cultured for mycobacteria when pleural tuberculosis is suspected, the culture yield of sputum specimens remains variable between 10 and 60%, predominantly determined by the extent of associated pulmonary involvement [13]. The isolation from pleural fluid samples also remains low and ranges between 60 and 70%. The probable justification of low culture yield could be the fact that the main pathogenesis in such cases is delayed hypersensitivity [14, 15]. Direct microscopy for presence of acid fast bacilli very rarely gives a positive result and with the exception of tubercular empyema or HIV co infection, the positivity often remains below 5–10% [7, 10]. Approximately one-third of patients with pleural tuberculosis give a negative skin test [15]. Most of the time empirical therapy is initiated on the basis of clinical suspicion as in almost 15% of cases no diagnosis is ever established [16]. With solid culture media, low sensitivities in the range of 12–30%

have been reported [12]. In our study 11.5, 15.4 and 3.8% of pleural fluid specimens were positive by direct microscopy for acid fast bacilli, culture and by both direct microscopy and culture respectively.

ADA besides being a key enzyme in purine catabolism, is also involved in catalysing the deamination of adenosine to inosine. ADA has been demonstrated in biological fluids in cases of infectious diseases primarily involving macrophages. In addition to that it also helps in the proliferation and differentiation of T-lymphocyte. Thus is an important indicator of cellular immunity [17]. There are numerous studies in the literature demonstrating increased levels of pleural fluid levels of ADA in the cases of tubercular pleural effusion. In our study the mean levels of ADA were 46.27 U/L in pleural tuberculosis cases. Our findings were in agreement with the majority of other studies which have used the cut-off of 40 U/L for the diagnosis of pleural tuberculosis [6, 18]. Though, wide range of cut-off values have been reported, the majority showing the most accurate threshold to range between 40 and 60 U/L [12]. Helmi et al. [6] reported a mean ADA level of 83.5 ± 50.3 U/L in tuberculous pleural effusion. Similarly, Garcia-Zamalloa et al. [18] have reported the mean pleural fluid levels of 56.7 U/L in their 10 years study in cases of tuberculous pleurisy. In a study involving 254 pleural tuberculosis cases, ADA was more than 47 U/L in 99.6% cases [19]. In another study conducted in a high TB prevalence population including 303 pleural tuberculosis cases with exudative effusions, 58% had ADA levels of more than 50 U/L [20].

A clinician need to be additionally aware of conditions while interpreting ADA levels for the diagnosis of pleural tuberculosis that may result in the increased likelihood of false results involving both the false-negative and false-positive ADA results. In the initial stages of disease the levels of ADA in the pleural fluid may be lower than the reference range that may lead to a false negative result. Moreover, false negative results may also be seen in elderly patients and smokers. There are several conditions which can lead to false positive results like rheumatoid effusion, empyema due to other bacteria, mesothelioma,

lung cancer and haematological malignancies. Measurement of ADA-1 isoenzyme of ADA can also produce false positive results as it is also increased in other bacterial empyemas. Thus the use of ADA-2 isoenzyme measurement increases the specificity for the diagnosis of pleural tuberculosis [12].

IFN- γ is a Th1 cytokine often assessed for host's immune response to mycobacterial infection. Though levels of pleural fluid IFN- γ concentration are considered a useful diagnostic tool to diagnose pleural tuberculosis, concerns regarding specificity have often been raised similar to ADA [21, 22]. Studies have reported cut off values of >140 to 240 pg/mL with sensitivity and specificity ranging from 89 to 95% and 92–97% respectively [19, 23, 24]. A study done in a TB endemic setting found a comparable potential utility of IFN- γ with ADA, though a significantly greater sensitivity of IFN- γ than ADA with a cut-off value of 107.7 pg/mL was reported. Yet definitive recommendations regarding routine use in clinical practice is lacking for both these markers as false positivity or false negativity concerns are there [25]. In our study we also analysed another Th1 cytokine of IL-2. The median serum level of IL-2 was found to be significantly higher in healthy controls than the pleural tuberculosis cases. Lower serum levels favoured the cases only for IL-2 among all the biomarkers under study. However, in a study by Li et al. [26] no statistical difference was observed for IL-2 between the three study groups of pleural effusion with pleural tuberculosis group, pulmonary tuberculosis cases group and healthy controls group. In the present study we found IFN- γ /IL-2 ratio as the best discriminatory biomarker with the highest AUC in ROC analysis among all the biomarkers under study. Despite our best efforts we could not find similar studies assessing the potential usefulness of IFN- γ /IL-2 ratio for the diagnosis of pleural tuberculosis. Neopterin is produced by activated macrophages in response to the action of interferon gamma. Thus, neopterin can be used as marker of macrophage activation and cellular immunity [27]. Increased levels of neopterin has been reported in various body fluids in tuberculosis cases [28]. In our study, we have demonstrated a median serum levels of 25.3 nmol/L. Baganha et al. [29] have also demonstrated increased neopterin levels in the sera of the tuberculous pleural effusion cases. Tozkoparan et al. [28] also demonstrated a mean serum neopterin level of $38.28 \text{ nmol/L} \pm 6.14$ which was significantly different from the control group ($p < 0.05$). In our study we have also reported a significant difference in the median serum levels of neopterin and healthy controls ($p < 0.001$). Many studies have reported increased pleural fluid neopterin levels in the tuberculous pleural effusion cases than the malignant pleural effusion cases [27, 29]. Immanuel et al. have demonstrated increased serum neopterin levels in

HIV-negative TB patients prior to the treatment. They further reported a decrease in the serum neopterin levels following first month of treatment and the levels returned to baseline values at the completion of full course of treatment. The same study also showed that increased serum neopterin levels after treatment were associated with relapse. Consequently, this study suggested that assessment of neopterin levels could be a useful marker for monitoring the response to treatment [30]. In this study we assessed serum levels of oxidative stress markers; protein carbonyl for amino acid oxidation and MDA for lipid peroxidation. Significantly raised median levels were obtained in the pleural tuberculosis cases when compared to the healthy controls. However, Gunes et al. [31] in their study reported no statistically significant difference in the serum MDA levels between tuberculous and non-tuberculous pleural effusion cases ($p = 0.526$).

Conclusion

In India, TB is the most common cause of pleural effusion. In a large number of cases diagnosis is made on the basis of a high ADA level in an exudative lymphocytic predominant pleural effusion. Though ADA is the most widely used biomarker for the diagnosis of tuberculous pleural effusion, correlation of ADA with the other conventional or newer methods is not well described. Moreover, false-negative and false-positive results warrant its cautious use. There is an urgent need for research in the field of biomarkers that are directly involved in disease pathogenesis or protection. Our study reports the preliminary observation of the diagnostic utility of T-cell function, macrophage activation and oxidative stress biomarker levels between TB—pleural effusion and healthy normals. The principle limitation of this study is the non-inclusion of disease control cases. Therefore further research involving larger and more diverse study groups particularly the disease control cases is necessary to validate these biomarkers under study for their potential diagnostic role in pleural tuberculosis.

Acknowledgements Dr. Rahul Sharma for statistical analysis and Dr. Rajat Jhamb for clinical specimens.

Compliance with Ethical Standards

Conflict of interest Author Nisha Goyal declares that she has no conflict of interest. Author Bineeta Kashyap declares that she has no conflict of interest. Author NP Singh declares that he has no conflict of interest. Author Iqbal R Kaur declares that she has no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964

Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

References

- World Health Organization. Global tuberculosis report 2016. Geneva: World Health Organization, 2016. <http://apps.who.int/iris/bitstream/10665/250441/1/9789241565394-eng.pdf?ua=1>.
- Govt. of India. Revised National TB Control Programme: Annual Status Report. Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi India. 2014. <http://www.tbcindia.nic.in/pdfs/TB%20India%202014.pdf>.
- Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res*. 2004;120(4):316–53.
- Solovic I, Jonsson J, Korzeniewska-Kosela M. Challenges in diagnosing extrapulmonary tuberculosis in the European Union, 2011. *Euro Surveill*. 2013;18:12.
- Biomarkers working group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69:89–95.
- Helmy NA, Eissa SA, Masoud HH, Ellessawy AF, Ahmed RI. Diagnostic value of adenosine deaminase in tuberculous and malignant pleural effusion. *Egypt J Chest Dis Tuberc*. 2012;61(4):413–7.
- Gopi A, Madhavan SM, Sharma SK, Shan SA. Diagnosis and treatment of tuberculous pleural effusion. *Chest*. 2006;131:880–9.
- Porcel JM. Tuberculous pleural effusion. *Lung*. 2009;187:263–70.
- Doosoo J. Tuberculous pleurisy: an update. *Tuberc Respir Dis*. 2014;76:153–9.
- Light WR. Pleural diseases. 5th ed. Baltimore: Lippincot, Williams and Wilkins; 2007.
- Stead WW, Eichenholz A, Straus HK. Operative and pathologic findings in twenty four patients with syndrome of idiopathic pleurisy with effusion presumably tuberculous. *Am Rev Tuberc*. 1955;71:473–502.
- Vorster MJ, Allwood BW, Diacon AH, Koegelenberg CFN. Tuberculous pleural effusions: advances and controversies. *J Thorac Dis*. 2015;7(6):981–91.
- Davies PD. Tuberculous pleuritis. In: Bouros D, editor. *Pleural disease*. New York: Marcel Dekker; 2004. p. 677–97.
- Ruan SY, Chuang YC, Wang JY, Lin JW, Chien JY, Huang CT, et al. Revisiting tuberculous pleurisy: pleural fluid characteristics and diagnostic yield of mycobacterial culture in an endemic area. *Thorax*. 2012;67:822–7.
- Porcel JM, Vives M. Differentiating tuberculous from malignant pleural effusions: a scoring model. *Med Sci Monit*. 2003;9:175–80.
- Light RW. Clinical practice. Pleural effusion. *N Engl J Med*. 2002;346:1971–7.
- Barua R, Hossain M. Adenosine deaminase in diagnosis of tuberculosis: a review. *Anwer Khan Mod Med Coll J*. 2014;5:43–8.
- Garcia-Zamalloa A, Taboada-Gomez J. Diagnostic accuracy of adenosine deaminase and lymphocyte proportion in pleural fluid for tuberculous pleurisy in different prevalence scenarios. *PLoS ONE*. 2012;7(6):e38729. doi:10.1371/journal.pone.0038729.
- Valdés L, Alvarez D, San José E, Penela P, Valle JM, García-Pazos JM, et al. Tuberculous pleurisy: a study of 254 patients. *Arch Intern Med*. 1998;158:2017–21.
- Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio. Increased specificity for the diagnosis of tuberculous pleuritis. *Chest*. 1996;109:414–9.
- Yurt S, Küçükergin C, Yigitbas BA, Seckin S, Tigin HC, Kosar AF. Diagnostic utility of serum and pleural levels of adenosine deaminase 1-2, and interferon- in the diagnosis of pleural tuberculosis. *Multidiscip Respir Med*. 2014;9:12.
- Aoe K, Hiraki A, Murakami T, Eda R, Maeda T, Sugi K, et al. Diagnostic significance of interferon-gamma in tuberculous pleural effusions. *Chest*. 2003;123:740–4.
- Wongtim S, Silachamroon U, Ruxrungtham K, Udompanicha V, Limthongkula S, Charoenlapa P, et al. Interferon gamma for diagnosing tuberculous pleural effusions. *Thorax*. 1999;54:921–4.
- Jiang J, Shi HZ, Liang QL, Qin SM, Qin XJ. Diagnostic value of interferon-gamma in tuberculous pleurisy: a metaanalysis. *Chest*. 2007;131:1133–41.
- Meldau R, Peter J, Theron G, Calligaro G, Allwood B, Symons G, et al. Comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN- γ for the evaluation of pleural tuberculosis: a prospective cohort study. *BMC Pulm Med*. 2014;14:58.
- Li YH, Xie CM. A study on the Th1/Th2 cytokines in the pathogenesis of human tuberculous pleuritis. *Zhonghua Jie He He Hu Xi Za Zhi*. 2004;27:324–7 (in Chinese, English abstract).
- Cok G, Parildar Z, Basol G, Kabaroglu C, Bayindir U, Habif S, et al. Pleural fluid neopterin levels in tuberculous pleurisy. *Clin Biochem*. 2007;40(12):876–80.
- Tozkoparan E, Deniz O, Cakir E, Yaman H, Ciftci F, Gumus S, et al. The diagnostic values of serum, pleural fluid and urine neopterin measurements in tuberculous pleurisy. *Int J Tuberc Lung Dis*. 2005;9:1040–5.
- Baganha MF, Mota Pinto A, Pego MA, Marques MA, Rosa MA, Cordeiro AJ. Neopterin in tuberculous and neoplastic pleural fluids. *Lung*. 1992;170:155–61.
- Immanuel C, Rajeswari R, Rahman F, Kumaran PP, Chandrasekaran V, Swamy R. Serial evaluation of serum neopterin in HIV seronegative patients treated for tuberculosis. *Int J Tuberc Lung Dis*. 2001;5(2):185–90.
- Gunes O, Erbaycu AE, Çakan A, Örmən M, Özsoz A, Önvural B. The diagnostic value of malondialdehyde level in pleural effusions. *Eurasian J Pulmonol*. 2003;5(4):213–9.