The Challenge of Diagnosing Pleural Tuberculosis Infection

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Tuberculosis (TB) remains a major threat problem and pleural TB is the most frequent extrapulmonary form of this disease. The diagnosis of TB involvement of the pleura is still challenging since clinical and imagistic findings are extremely non-specific. Clinical findings, chest X-ray, CT scan and simple pleural fluid analysis may be suggestive but do not confirm the diagnosis of pleural TB. Tuberculin skin test is often negative. Identification of the mycobacteria by culture is difficult and the results from sputum and pleural fluid are often negative. Recent advances such as the BACTEC system and the polymerase chain reaction (PCR) have increased the rate of mycobacteria identification. Among the new biomarkers, the most promising are adenosine-deaminase (ADA) and interferon-gamma (IFN-gamma). In certain circumstances, the high levels of ADA and/or IFN-gamma may allow the clinician to start specific TB therapy in the absence of a confirmation by cultures or biopsy. Pleural biopsy (blind percutaneous or through thoracoscopy) remains an option for cases which are not elucidated by less invasive methods.

Keywords pleural effusion; Mycobacterium tuberculosis; diagnosis

1. Introduction

Tuberculosis remains a major health threat, being responsible for almost 1.5 million deaths every year [1]. Pleural TB is the most frequent extrapulmonary form, being commonly encountered in the clinical practice, especially in areas with high incidence of TB infection. Due to the specific pathogenesis of the pleural TB effusions, which is related more to an immune response than to a direct mycobacterial involvement, the diagnosis is still difficult and controversial [2- 7]. Proving the TB etiology of a pleural effusion is very important since in the absence of a correct diagnosis there will be no adequate treatment, leading eventually to relapse and development of severe complications, some of them requiring prolonged treatments or even major and/or mutilating surgical procedures [8-10].

2. Clinical manifestations may suggest the diagnosis of pleural TB but they are extremely non-specific, even in endemic areas.

Most patients have an acute onset (one week - one month) with:
-cough (70%) which is usually non-productive;
-chest pain (70%) which is usually a pleuritic one;
-fever (85%) [2, 3].
   A particular aspect that should raise the suspicion of possible TB is poor response to treatment with usual antibiotics [4].
   In some patients the onset is less acute with mild chest symptoms associated with weight loss, fatigability and an overall malaise. HIV patients present more often systemic symptoms but it is difficult to determine if they are due to the pleural TB or to the HIV infection [11].
   Dyspnea depends on the previous respiratory status and the volume of the pleural effusion, which may be of any size. To note that massive effusions are not uncommon, being encountered in almost 20% of the cases; emergency admission for severe dyspnea due to a massive pleural TB effusion is a frequent scenario in the regions with a high incidence of TB [3, 12].

3. Imagistic findings

Chest X-ray, ultrasound and CT scan may show the presence of the pleural liquid, which does not have any particular aspect useful for the diagnosis of TB. As a general rule, ultrasound and CT may detect smaller amounts of liquid compared to plain chest X-ray [13, 14].
   Chest X-ray and CT scans may detect parenchymal lesions highly suggestive for TB in up to 40% of the cases, with no clear relationship between the degree of the parenchymal damage and the pleural inflammation [15].

4. Pleural fluid analysis

Is mandatory in any patient with a pleural effusion of unknown etiology. Although it gives a definitive diagnosis only in rare occasions, it gives several important clues that can help the clinician in guiding the future investigations [16]. The most important characteristics are:

-exudative nature;

-high protein levels, often > 5g/dl;

-high levels of lymphocytes (usually > 50%, often with higher levels; the proportion of lymphocytes is < 50% in less than 5-10 % of the patients with pleural TB).

-at the beginning of the disease (symptoms for less than 2 weeks) the differential white blood cells count may show predominantly polymorphonuclear leukocytes; in time, if repeated thoracentesis and pleural fluid analyses are performed, the proportion changes in the favour of the small lymphocytes.

The aforementioned characteristics do not support a definitive diagnosis of pleural TB but are highly suggestive. If this characteristics are used together with other tests (i.e. lymphocytic effusion with high ADA levels), in certain circumstances they have an accuracy that is high enough to allow starting of specific antiTB therapy without positive cultures or biopsy [2, 3].

Other diagnostic clues are:

-the level of eosinophils: if they exceed 10% the diagnosis of pleural TB is very unlikely unless the patient has a history of recent pneumothorax or thoracentesis [2, 17].

-the level of mesothelial cells, which rarely exceed 5% in pleural TB. A level of mesothelial cells < 5% is not very useful since this is characteristic to any disease that involves an intense inflammation of the pleural surfaces. However, a high level of mesothelial cells makes the diagnosis of pleural TB very unlikely [18], the only notable exception being pleural TB developed in HIV patients, usually with low CD4 counts [19].

5. Tuberculin skin test

Is a very useful test if the reaction is positive. The main problem with tuberculin skin test in pleural TB effusions is that it is often negative – at least in 40-50% of the cases [20]. However, if it is performed after at least 8 weeks from the development of the symptoms, the tuberculin skin test becomes almost always positive. Therefore, the tuberculin skin test is useful in the following clinical circumstances [2]:

-if positive, it is highly suggestive for the TB etiology of the pleural effusion;

-if it remains negative at 8 weeks after the first symptoms, it can be used to exclude TB.

To note that in immunosuppressed patients such as those with HIV or severe malnutrition, the tuberculin skin test remains often negative [21].

6. Examination of the sputum for mycobacteria (smears and cultures)

The sputum should be examined for mycobacteria in any patient with a pleural effusion of unknown etiology, although it is frequently overlooked, especially if the patient does not have parenchymal lesions suggestive for TB. Several studies report that smears and cultures of the patients with pleural TB are positive in up to 22-52% [2, 22, 23]. The great differences reported in the literature may be explained by different patterns of TB endemy and the incidence of HIV and of the associated parenchymal lesions.

7. Examination of the pleural fluid for mycobacteria (stains and cultures)

The examination of the pleural fluid for the presence of mycobacteria is mandatory in patients with pleural effusions of unknown etiology [3]. Pleural smears are almost always negative, unless the patient is HIV positive. Published results are quite variable, with recent studies showing rates of positive cultures up to 50-60% [24, 25]. Although the rate of positive results is still low and it takes time to achieve the final result, pleural cultures may clarify the diagnosis in certain situations. Another major advantage is that this is the only way to obtain information about the sensibility of the involved mycobacteria to the specific anti TB drugs [6].

Recent studies have shown that bedside inoculation [26, 27] and the use of the BACTEC system instead of the classic Löwenstein-Jensen medium [28] allows improved results by:

-achieving higher rates of mycobacteria detection;

-accelerating the diagnosis by 2-3 weeks.

8. Polymerase chain reaction (PCR)

Is seen as a promising diagnostic tool for pleural TB by some authors. Several studies have shown that the PCR analysis of the pleural liquid offered higher rates of identifying mycobacteria than the acid fast bacilli stains and cultures, even compared with the BACTEC system [29, 30]; PCR remained superior also in HIV with pleural TB [31]. Another obvious advantage is related to the fact that PCR offers a positive or negative result much faster compared to the classic cultures which may take up to 4-6 weeks.

In a recent study, Kalantri et al. (2011) found that the combination of either real-time PCR or IFN-gamma showed a sensitivity of 100% in confirmed pleural TB and 96.2% in probable pleural TB, but the accuracy of PCR alone was lower than that of IFN-gamma or ADA [32]. A study published by Kumar et al. (2010) showed a significant increase of sensitivity when performing PCR assay on both sputum and pleural liquid [33]. The disparate results reported by several authors may be explained by the different technology used.

However, since PCR alone does not seem to have a much higher accuracy compared to the much simpler ADA determination [32, 34], its role in the diagnosis of pleural TB remains to be determined.

9. Adenosine deaminase, interferon gamma and other biological markers

9.1. Adenosine-deaminase (ADA)

Is a predominant T-lymphocyte enzyme that catalyzes the conversion of adenosine to inosine; its activity is raised in diseases which are associated with a stimulation of the cellular immunity. Since pleural TB results in an increased activity of the monocytes and macrophages, this will lead to an increased ADA level in the pleural fluid [2, 3, 7].

In the early 1970's, Piras and Gakis described elevated levels of ADA in the cerebrospinal fluid of the patients with TB meningitis, followed by the investigation of ADA levels in the pleural fluid [35]. Since then, a lot of studies have shown high levels of ADA in the pleural fluid of the patients with pleural TB, showing a diagnostic sensitivity between 77-100% and specificity between 81-97% [36-43].

Other diseases with high ADA levels in the pleural fluid are [44]:

-empyema and collagen diseases, especially rheumathoid polyarthritis, both of them without a high level of lymphocytes;

-malignant lymphoma, which may occasionally present both high ADA and lymphocytes/neutrophiles ratio in the pleural fluid, but with particular lesions on CT and PET scans.

Due to its high accuracy and availability, the determination of the ADA level in the pleural is now mandatory if TB is suspected or if we want to rule out this diagnosis [3, 6]. The cut-off level is not clear, since different values were used by different authors, usually in a range between 40 and 71 U/l [7].

From the practical point of view it should be remembered that:

-the chance to have TB pleurisy increases with the value of ADA level in the pleural fluid, being:

-almost zero for levels below 40 U/l;

-almost 100% for levels over 70 U/l if empyema, collagen diseases and lymphoma are excluded;

 -the chance to have TB pleurisy in a patient with a high ADA level is also increased if:

-the pleural fluid is rich in lymphocytes, with a lymphocytes/neutrophils ratio > 0.75;

-the patient comes from an endemic TB area;

-the patient is young [36, 37, 45].

-last but not least, the ADA levels in the pleural fluid can be determined very quick and easy compared with the time required for the cultures, even when using modern techniques.

Other advantages of the use of ADA are:

-the ADA pleural fluid levels remain high in HIV patients with TB [46], even if the CD4 levels are low [47];

-the ADA levels remain stable for a long time if preservatives and/or low temperature are used. Therefore, if ADA levels cannot be determined in a certain hospital, the probes can be easily send to distant laboratories [48, 49].

ADA1 and ADA2 are two isoenzymes who give the total ADA level by summation. ADA1 can be found in almost any cell but the greatest activity is in lymphocytes and monocytes, while ADA 2 is found only in monocytes. In pleural TB, most of the ADA level is given by the ADA 2 isoenzyme [50, 51]. Therefore, it is suggested that an ADA1/total ADA level < 0.42 increases the diagnostic accuracy of the pleural fluid ADA level [52], but separate determination of the ADA isoenzymes is not routinely used by most clinicians [3, 4, 6].

9.2. Interferon gamma (IFN-gamma)

IFN-gamma is produced by the activated CD4+ lymphocytes from the patients with TB effusions. Several studies have shown that the levels of IFN-gamma are higher in patients with TB compared with other etiologies of the pleural effusions but a clear interpretation of the data is difficult due to different cut-off values and laboratory methods used to measure the IFN-gamma levels. Most of the false positive results are related to hematologic malignancies and empyema

[2, 32, 53-55]. Jiang and colleagues (2007) performed a metaanalysis and found an overall sensitivity of 89% and a specificity of 97% [56].

9.3. ADA vs. IFN-gamma.

Several studies have compared ADA and IFN-gamma and found no major differences between the two tests [32, 54, 55, 57, 58]. The main disadvantage of both ADA and IFN-gamma is their inability to provide information about the degree of resistance to the antiTB drugs [6]. Since ADA determination is significantly cheaper and easier to perform, it is preferred by most of the clinicians [3].

9.4. Other tests.

Different biological markers have been investigated in pleural TB in order to avoid the use of invasive biopsy methods.
  -lysozyme - an enzyme found in the granulomatous epitheloid cells and activated macrophages, but also in some tumor cells [59, 60];
  -tuberculostearic acid - a structural component of the mycobacteria and actinomycetes [61, 62];
  -antimycobacterial antibodies such as the anti-P32 [63] and different monoclonal antibodies [64];
  -different cytokines such as interleukines, TNF alpha, VEGF etc [65, 66].
  Different studies have shown elevated levels of the aforementioned biological markers in case of pleural TB but none of them has been shown to have a higher performance compared with the determination of ADA and IFG; also, some of them require more complicated and expensive technologies. These biological markers are now investigated but most of them are not routinely recommended in clinical practice [3].

10. Pleural biopsy

Is a classic method to establish the diagnosis of pleural TB by pathologic demonstration of typical granuloma. In our days, it is used less frequently – mainly in patients with pleural effusions of unknown etiology, where other less invasive tests were not able to clarify the diagnosis. There are 3 ways to obtain a pleural biopsy specimen:
  -blind percutaneous biopsy: this is the less invasive method, although it is not without complications; in most of the large series dedicated to this diagnostic procedure, a great proportion of the patients were diagnosed with pleural TB [67-69].
  -thoracoscopy: is more and more accepted as a diagnostic tool for pleural diseases [70-72]. In some circumstances such as a loculated effusion, it is not only a diagnostic, but also a therapeutic tool by performing a decortication and an adequate drainage [73].
  -open thoracotomy: this is no longer accepted as a diagnostic method. However, if the patient undergoes a thoracotomy to solve a complicated pleural disease, pleural biopsy is mandatory. From our experience, it is not unusual to diagnose TB based on open pleural biopsy in patients with previous negative tests for mycobacterium tuberculosis, especially if the patient had a complicated course [10, 74].
  The choice between blind percutaneous or thoracoscopic biopsy is still a matter of debate and depends on more factors [75-76]:
  -thoracoscopy allows a clear visualization of the pleural surfaces, but because of the uniform involvement of the pleura this aspect is less relevant in pleural TB (opposed to malignant effusions where the chance to miss the metastatic deposits by a blind biopsy is higher);
  -if the patient has a loculated effusion, thoracoscopy allows breaking of the adhesions and an adequate lavage and drainage;
  -blind biopsy is less expensive and much more available than thoracoscopy; this aspect is important since the incidence of TB is higher mainly in poor income countries, where the availability of thoracoscopy is lower.
  If a pleural biopsy is performed, the specimen should be sent not only for pathological examination, but also at least for acid fast bacilli stain and culture for Mycobacterium tuberculosis. These two tests are more often positive compared with the same tests performed from the pleural liquid [3]. In a study performed by Valdes and colleagues (1998) on 254 patients with pleural TB who underwent pleural biopsy they found that 80% showed typical granulomas, 25.8% had positive acid fast bacilli stains and 56% had positive cultures from the biopsy specimens; overall, at least one of the three tests was positive in 91% of the cases, with some patients presenting no granulomas but positive acid fast bacilli stains or cultures from the pleural biopsy [20].
  Some authors suggest that the pleural biopsy specimens should be sent for even more complicated tests – including PCR or DNA identification [72, 77].

11. Conclusions

The diagnosis of pleural TB involvement remains a challenge but significant improvements have been made during the last years. Classic diagnostic tools based on mycobacteria identification from sputum or pleural fluid have a low rate of positive results, which is improved by modern techniques based on BACTEC and PCR. New biomarkers such as ADA and IFG-gamma may in certain circumstances allow starting the antiTB treatment, even in the absence of culture or pathologic confirmation of the diagnosis. Biopsy remains an option for unresolved cases but it is rarely performed due to the increased accuracy of the modern tests. Clinical judgment and correlation between clinical, imagistic and laboratory data remain essential.

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