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REVIEW

Novel tests for diagnosing tuberculous pleural effusion: what works and what does not?

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ABSTRACT: Tuberculous pleuritis is a common manifestation of extrapulmonary tuberculosis and is the most common cause of pleural effusion in many countries. Conventional diagnostic tests, such as microscopic examination of the pleural fluid, biochemical tests, culture of pleural fluid, sputum or pleural tissue, and histopathological examination of pleural tissue, have known limitations. Due to these limitations, newer and more rapid diagnostic tests have been evaluated. In this review, the authors provide an overview of the performance of new diagnostic tests, including markers of specific and nonspecific immune response, nucleic acid amplification and detection, and predictive models based on combinations of markers. Directions for future development and evaluation of novel assays and biomarkers for pleural tuberculosis are also suggested.

KEYWORDS: Biomarkers, diagnosis, pleuritis, sensitivity, specificity, tuberculosis

Tuberculous pleuritis is a common manifestation of extrapulmonary tuberculosis (TB) and is the most common cause of pleural effusion in many countries [1–3]. Pleural TB occurs as a result of a TB antigen entering the pleural space, usually through the rupture of a subpleural focus, followed by a local, delayed hypersensitivity reaction mediated by CD4+ cells [4]. This process may occur during primary or reactivation TB and may or may not involve viable bacilli entering the pleural space [5].

The presence of mycobacterial antigens in the pleural space elicits an intense immune response, initially by neutrophils and macrophages [6, 7], followed by interferon (IFN)-γ-producing T-helper cell (Th) type 1 lymphocytes [4, 8], resulting in a lymphocyte-predominant exudative effusion. This cellular trafficking is facilitated by homing surface markers and chemokine gradients [9, 10]. This intense but poorly understood local immune response by sensitised lymphocytes to TB antigen is synonymous with the Koch phenomenon [11]. A recent study indicates that cells of an alternative T-cell profile, CD4(+)/CD25(+)/FoxP3(+) regulatory T-cells, are expanded in TB pleural effusion and can suppress some effector responses, although the precise role of these cells remains unclear.

Conventional diagnostic tests for pleural TB include microscopic examination of the pleural fluid for acid-fast bacilli, mycobacterial culture of pleural fluid, sputum or pleural tissue, and histopathological examination of pleural tissue for granulomatous inflammation. These tests have recognised limitations for clinical use, although, in combination, they have been recognised as the best reference standard for evaluation of the accuracy of novel tests [12].

Microscopy of the pleural fluid for acid-fast bacilli is positive in <5% of TB pleuritis cases, due to the paucibacillary nature of the disease [12, 13]. Mycobacterial culture of pleural fluid also has low sensitivity (24–58%) [13, 14] and is limited by the lengthy delay in obtaining results: up to 8 weeks if solid culture media are used. Biopsy of pleural tissue for combined histological examination and mycobacterial culture of pleural fluid and tissue is the most sensitive of the currently available diagnostic methods, but may still be falsely negative in 15–20% of these cases [12, 15]. In addition, pleural biopsy is invasive, and yield as well as complication rates are dependent on the skill of the operator because it is technically difficult, particularly in children. Hence, biopsy adds considerable risk and cost to

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the workup. Mycobacterial culture of spontaneous sputum or gastric lavage has a variable yield, from 0% [16] to ~30% [17], depending on the presence of associated lung parenchymal lesions [17]. Since patients with pleural TB rarely produce sputum spontaneously, routine collection of induced sputum has been proposed for the diagnosis of pleural TB. In a prospective study of 113 patients with confirmed pleural TB, induced sputum had a sensitivity of 52%, compared with 12% sensitivity of pleural fluid culture [18]. Due to these limitations of conventional tests, plus the delay of several weeks for mycobacterial culture results, newer rapid tests and biomarkers have been evaluated. The present review provides a narrative overview of the literature on the tests, summarised in table 1 and illustrated in figure 1, and reviews their performance characteristics for the diagnosis of pleural TB.

For clarity, all tests were grouped into the following categories, although some overlap exists: 1) nonspecific inflammatory and immune response markers; 2) specific markers of immune response; 3) nucleic acid amplification tests; and 4) scoring systems based on combinations of tests.

NONSPECIFIC INFLAMMATORY AND IMMUNE RESPONSE MARKERS

Adenosine deaminase

Adenosine deaminase (ADA), released by activated lymphocytes, macrophages and neutrophils, is a nonspecific marker of inflammation. The ADA2 isoenzyme released from monocytes and macrophages is the predominant contributor to total ADA activity [49]. A high diagnostic accuracy of ADA activity measurement has been reported in several studies. In a meta-analysis, GRECO *et al.* [19] found that among 31 studies published prior to 2000, including 4,738 patients, the pooled sensitivity was 92% (range 56–100%) and pooled specificity was 89% (55–100%) using composite reference standards including culture, histology, sputum culture and response to therapy. The specificity for discriminating TB from malignant effusions, an important differential diagnosis in elderly patients, remained high (95%) but was disappointingly low for parapneumonic effusions.

GRECO *et al.* [19] concluded that in low and intermediate TB incidence settings, the negative predictive value was sufficient that a negative ADA activity result would preclude the need for pleural biopsy. However, in the same settings, the positive predictive value was poor. In contrast, in high prevalence settings, a positive ADA would provide a 99% post-test probability of TB. This crucial point, that the predictive value of a test is highly dependent on the prevalence of the disease, explains the differences in performance of this test in different studies. Thus, the clinical context must be taken into account when interpreting the ADA result.

Many studies in the review by GRECO *et al.* [19] included <30 patients, resulting in wide confidence intervals (CIs) around sensitivity and specificity estimates and significant heterogeneity

TABLE 1 Novel tests and biomarkers for the diagnosis of tuberculous pleural effusions

Test or biomarker	Performance characteristics	[Ref.]
Nonspecific inflammatory and immune response markers		
ADA activity	Consistent high sensitivity and reasonable specificity	[19–23]
	Inexpensive, simple, rapid	
	Best performance among nonspecific inflammatory markers	
Neopterin	Limited data, variable sensitivity and specificity	[24–27]
Leptin	Limited data	[28]
Lysozyme	Low specificity	[29, 30]
Fibronectin	Limited data, comparison with malignant effusion only, no established cut-off	[30]
IFN-γ measurement	Consistent high sensitivity and specificity, kit available, expensive	[19, 31, 32]
	Best performance of the group	
IL-2	Lower sensitivity and specificity than IFN-γ	[33]
TNF-α, IL-1β	Limited data, no established cut-off	[34]
Complement activation	Intermediate sensitivity and specificity, better performance in diagnosis of rheumatic diseases and parapneumonic effusions	[35–37]
CD4+ T-cell count	Limited value for TB diagnosis	[38]
Specific markers of immune response		
T-cell response to ESAT-6 and CFP-10	Limited data, few patients, specificity data needed	[39]
Serologic (antibody) tests	Very low sensitivity, high specificity, best sensitivity with an in-house method detecting antibodies against MPT-64 and MT-10.3	[20, 40–47]
	High specificity with TBGL antigen	
	Other antigens (P32, A60) disappointing	
Detection of *Mycobacterium tuberculosis* nucleic acid by commercial NAATs	Low and variable sensitivity, high specificity, kits not approved for extrapulmonary specimens	[48]

ADA: adenosine deaminase; IFN: interferon; IL: interleukin; TNF: tumour necrosis factor; ESAT: early secreted antigenic target; CFP: culture filtrate protein; NAAT: nucleic acid amplification test; TB: tuberculosis; TBGL: tuberculous glycolipid.

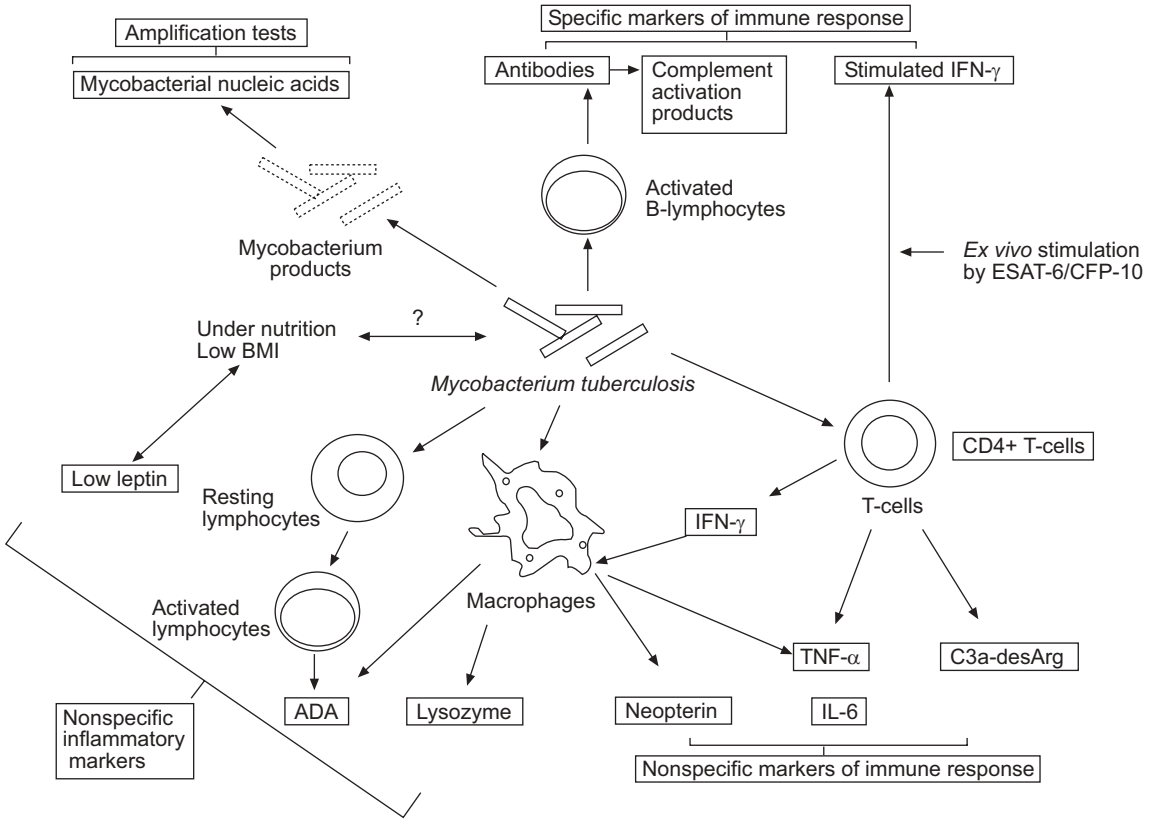

FIGURE 1. Schematic representation of pathways and systems involved in biomarkers for pleural tuberculosis. IFN: interferon; ESAT: early secreted antigenic target; CFP: culture filtrate protein; BMI: body mass index; ADA: adenosine deaminase; TNF: tumour necrosis factor; IL: interleukin.

between estimates. In addition, few studies reported blinding or described in detail the inclusion criteria for the control group.

Using histopathology as the reference standard, pleural fluid ADA activity, PCR and immunoglobulin (Ig)A-ELISA tests were evaluated in a high TB incidence country [20] among 77 patients with pleural effusion, 60 of whom had TB pleuritis. ADA activity was the only test that had a significantly higher sensitivity than histopathological examination. The study included only 17 nontuberculous effusions, and, therefore, specificity could not be adequately estimated [21].

Determination of ADA isoenzyme activity in pleural fluid may increase the accuracy of the test. ADA1 is secreted by lymphocytes and monocytes, while ADA2 is secreted only by monocytes and is found in a higher concentration in TB pleuritis [22, 23]. However, because the additional yield is small, a study would require a very large sample size to demonstrate that isoenzymes have significantly higher specificity than total ADA activity.

Neopterin

Neopterin is a marker of Th1 immune activation, as it is secreted by activated macrophages. Neopterin levels in pleural fluid have been found to be higher in patients with TB than patients with malignancy [24, 25] or other [26] conditions. Very high levels of neopterin have been observed in uremic pleural effusions [27], raising doubts about its specificity for TB. In one study that used histological evidence of caseating granuloma as the reference standard for TB, the sensitivity and specificity of neopterin were 44 and 85%, respectively [25]. In a second study, which used ADA as the reference standard, the sensitivity was 85% and the specificity was 93% [26]. However, ADA may not be a suitable reference standard because it is also an indicator of nonspecific inflammation, so a high degree of correlation may reflect the same underlying inflammatory response as neopterin.

Leptin

Leptin, a 16-kDa product of the obese (ob) gene, may be involved in cross-regulation between nutritional status and the immune response in TB. Serum leptin levels have been shown to be reduced in patients with active pulmonary TB [50] and cancer [51]. One study evaluated total leptin pleural fluid levels and leptin pleural fluid/serum ratio in 17 patients with pleural TB, and parapneumonic (n=7) and malignant (n=21) effusions [28]. The reference standard for TB in this study was the presence of granuloma in pleural tissue. ADA activity was also measured in pleural fluid. Pleural ADA activity sensitivity and specificity were both 100% and pleural leptin (<9.85 ng·mL^{-1}) sensitivity and specificity were 82% ($p=0.01$). Thus, there is no evidence that leptin performs better than pleural fluid ADA.

Lysozyme

Lysozyme is present in epithelial cells of granulomas, macrophages and activated granulocytes. In a study of patients with pleural effusion in a setting with high TB incidence, VALDÉS *et al.* [29] measured ADA activity in 405 specimens, lysozyme in

276 specimens and IFN-γ in 145 specimens. Using culture of pleural fluid or tissue and histopathological evidence of caseous granuloma in pleural tissue as the reference standard, the sensitivity of ADA, lysozyme and IFN-γ were 100, 85.7 and 94.2%, respectively, while specificities were 94.9, 61.6 and 91.8%, respectively. Thus, ADA and IFN-γ were more accurate than lysozyme levels. However, 51 patients were excluded from the analysis because a final definitive diagnosis could not be achieved, and the criteria for selection of specimens for lysozyme and IFN-γ testing were not reported. For these reasons, potential selection bias cannot be excluded.

MORIWAKI *et al.* [30] found that lysozyme could discriminate malignant from tuberculous effusions with a sensitivity of 100% and a specificity of 83%, compared with culture and/or histopathology of pleural tissue. The pleural fluid/serum lysozyme ratio had a sensitivity of 100% and a specificity of 88%. The mean fibronectin concentration in pleural fluid with tuberculous effusion was significantly higher than that in malignant effusion, but there was a marked overlap between the groups. In this study, lysozyme and fibronectin were less accurate than pleural fluid ADA [30].

Cytokines

Of all the cytokines, IFN-γ has been the most studied. The evidence for use of IFN-γ in pleural fluid for diagnosis of TB has been reviewed by GRECO *et al.* [19] and JIANG *et al.* [31]. In the latter review, based on 22 studies including 782 TB patients and 1,319 non-TB patients, the summary estimate of sensitivity was 89% (95% CI 87–91%) and of specificity was 97% (96–98%) [31]. IFN-γ was more sensitive and specific for the diagnosis of pleural TB than interleukin (IL)-12p40, IL-18, immunosuppressive acidic protein or soluble IL-2 receptor, in a study that directly compared these tests in the same samples [33]. In another study, the accuracy of IFN-γ was superior to that of ADA [19].

The concentration of other pro-inflammatory cytokines, such as tumour necrosis factor-α and IL-1β, has been shown to be higher in tuberculous than in malignant effusions, but no cut-off value for positivity has been established [34]. In another study, IL-8 and IL-6, and soluble IL-6 receptor were detected in carcinomatous but not in tuberculous pleural effusions [52].

Complement activation

PORCEL *et al.* [53] measured SC5b-9 and C3a-desArg, two products of complement activation, in the pleural fluid of 83 patients using commercial ELISA tests. The sensitivity was 84% for SC5b-9 and 81% for C3a-desArg. HARA *et al.* [35] showed SC5b-9 had a higher sensitivity (100%) and ADA activity had a higher specificity than conventional tests (culture and/or histopathological examination). Discrimination of TB pleuritis from malignant effusions was better than discrimination from autoimmune or parapneumonic effusions. In fact, the SC5b-9 level seemed more useful for the diagnosis of rheumatoid pleural effusions [36] and for differentiation of empyema from uncomplicated parapneumonic effusions [37].

Cell subsets

Tuberculous effusions are usually rich in mononuclear cells [12]. In one study, although pleural fluid ADA activity correlated with the number of CD4+ T-cells in the pleural space [38], the number of pleural CD4+ cells was not an accurate diagnostic test for TB.

In conclusion, among the nonspecific inflammatory biomarkers that have been evaluated, ADA and IFN-γ are the most accurate for pleural TB diagnosis, with consistently high sensitivity in many studies. Specificity of ADA is a concern because of the nonspecific nature of inflammatory response. Most studies used in-house methods for ADA activity estimation, which may demonstrate considerable variability in performance. Commercial kits have been used in a few studies, but these kits should be validated in different settings. ADA activity measurement is simple and inexpensive to perform and does not require special equipment, making it an attractive option for settings with high TB incidence and limited resources. In contrast, measurement of IFN-γ is expensive, estimated to be as much as the cost of treatment for six TB patients [32].

SPECIFIC MARKERS OF IMMUNE RESPONSE

T-cell response to specific antigens

Recently, *in vitro*, T-cell-based IFN-γ release assays (IGRAs) have been developed and licensed for diagnosis of latent TB infection. Normally, these tests use peripheral blood mononuclear cells (PBMCs), but they can be used with pleural fluid mononuclear cells. These assays detect IFN-γ secreted by mononuclear cells in response to *in vitro* stimulation with the *Mycobacterium tuberculosis*-specific antigens early secreted antigenic target-6 and culture filtrate protein-10. The genes that encode these antigens are not present in any of the *M. bovis* bacille Calmette–Guerin (BCG) strains or certain common nontuberculous (environmental) mycobacteria [54]. Thus, in theory, the test should not cross-react with antigens present due to BCG vaccination [54, 55]. In a recent study [39], the T-SPOT.*TB* test (Oxford Immunotec Ltd, Oxford, UK) was performed on PBMCs and mononuclear cells from pleural fluid from 20 patients clinically suspected to have TB pleuritis and 21 subjects with other diagnoses. The sensitivity of T-SPOT.*TB* was 90% using the blood assay and 95% for pleural fluid, but the specificity was only 67% for blood and 76% for pleural fluid. This poor specificity may reflect positive reactions due to coincidental latent TB infection, coexisting or transient infection.

An alternative commercially available T-cell assay using an ELISA platform is the QuantiFERON®-TB Gold in-tube test (Cellestis Ltd, Carnegie, Australia), which is more amenable to high throughput and flexibility with analysis. However, unpublished preliminary observations indicate that high background IFN-γ levels in the negative control tube precludes the use of *ex vivo* unprocessed pleural fluid in the assay. Prespecified numbers of mononuclear cells in culture medium may overcome this drawback.

IGRAs, designed to detect latent TB infection, will only be useful for the diagnosis of pleural TB disease if it can be shown that persons with latent TB infection and pleural effusions due to nontuberculous causes, such as cancer, have positive IGRA using serum, but negative IGRA using pleural fluid. This finding would suggest that TB-sensitised lymphocytes remain in peripheral blood but are not present among the lymphocytes found in the pleural fluid. This hypothesis as yet remains

unproven. Until this issue is settled, these assays may not offer any additional value for the diagnosis of pleural effusion, beyond the measurement of free IFN-γ in the pleural fluid.

B-cell response (antibody detection)

Although detection of serum antibodies against TB antigens is known to have poor and highly variable sensitivity and specificity [56, 57], attempts have been made to detect antibodies in pleural fluid specimens using techniques such as ELISA. Among pleural fluid antibody tests, detection of IgA against MPT-64 and MT-10.3 (Rv3019c), two recombinant protein antigens, had the best sensitivity [20, 40]. Anti-A60 IgM and IgG antibodies in pleural fluid had suboptimal sensitivity for TB [41]. Using antibodies against five different antigens, CHIERAKUL *et al.* [42] found the performance of serology of pleural fluid to be disappointing, with sensitivity and specificity <60%. Anti-P32 levels were reported to be higher among five patients with pleural TB, but the sensitivity and specificity were not reported [43]. Sensitivity to anti-lipoarabinomannan (LAM) and to anti-TB glycolipid antibodies was very low in other studies, although specificity was high [44–47].

One study reported a simple and cost-effective diagnostic tool (an in-house TB screen test) for pulmonary and extrapulmonary TB, using a liposome agglutination assay to detect serological responses to purified mycobacterial glycolipid antigens [58]. The assay was able to detect very low anti-glycolipid antibody concentrations in the serum of individuals with TB. The status for latent TB in the control group was not reported. To date, the test has not been applied to pleural fluid specimens, although four subjects with pleural TB were serum tested [58].

In conclusion, serologic tests using antibodies against mycobacterial protein and glycolipids show some potential for the diagnosis of pleural TB because of their high specificity, but are limited by very poor sensitivity.

DETECTION OF *M. TUBERCULOSIS* NUCLEIC ACID SEQUENCES BY AMPLIFICATION TESTS

Several commercial and in-house assays exist for the amplification and detection of *M. tuberculosis* nucleic acids from specimens such as sputum. These tests have also been used with specimens such as pleural fluids. In a meta-analysis of 40 studies of nucleic acid amplification tests (NAATs) for pleural TB, PAI *et al.* [48] reported that commercial NAATs have a potential role in confirming TB pleuritis because of high specificity (98 (95% CI 96–98)%). However, these tests had low and variable sensitivity (62 (43–77)%) and, therefore, were not useful in excluding the disease. The accuracy of in-house NAATs was poorly defined because of heterogeneity across studies, presumably reflecting the heterogeneity of in-house test protocols. Similar results have been reported with NAATs for TB meningitis [59] and TB lymphadenitis [60].

The reasons for the low sensitivity of NAATs in pleural fluid specimens are unclear. The presence of inhibitory substances in pleural fluid is an unsatisfactory explanation, as most commercial assays have an internal positive control to account for inhibition. The small amount of mycobacteria in pleural effusion may play some role, but the low sensitivity is more likely to be related to a technical aspect of nucleic acid extraction. Thus, caution is needed when interpreting negative NAAT results in pleural fluids, and performance will depend largely on pre-test probability.

In conclusion, the evidence is consistent that NAATs have modest sensitivity but excellent specificity for pulmonary and extrapulmonary TB. At present, no commercial kit has been approved by the US Food and Drug Administration for the diagnosis of extrapulmonary TB, and NAATs cannot be used in isolation to rule in or rule out pleural TB.

SCORING SYSTEMS BASED ON COMBINATIONS OF MARKERS

Due to the limitations of individual tests, scoring systems have been developed, based on the results of multiple tests (table 2). A scoring system that makes use of simple clinical and pleural fluid data was created by PORCEL and VIVES [61] to discriminate between TB and malignant pleural effusions. Two models were developed, one with and one without ADA activity measurement. In the first model, four variables predicted a tuberculous aetiology: ADA ≥ 40 U·L^{-1} (five points); age <35 yrs (two points); temperature $\geq 37.8^{\circ}$C (two points); and pleural fluid red blood cell count $<5\times10^{9}$·L^{-1} (one point). In the second model, TB was predicted by: age <35 yrs (two points); temperature $\geq 37.8^{\circ}$C (two points); pleural fluid red blood cell count $<5\times10^{9}$·L^{-1} plus no history of malignancy (three points); pleural protein ≥ 50 g·L^{-1} (one point); and pleural fluid/serum lactate dehydrogenase ratio ≥ 2.2 (one point). Summated scores of at least five in model one and at least six in model two yielded measures of sensitivity (95 and 97%, respectively) and specificity (94 and 91%, respectively) for discriminating TB from malignant effusions.

VILLEGAS *et al.* [62] also proposed a combination of clinical and biological markers. In their study, ADA activity, IFN-γ levels

TABLE 2 Combination of tests and biomarkers for the diagnosis of tuberculous pleural effusions

Combinations of markers	Reported accuracy	[Ref.]
Age + fever + red blood cells + ADA	Very high sensitivity, high specificity	[61]
ADA + IFN-γ + NAAT	Increase in sensitivity and specificity compared with each separate method	[62]
Duration of symptoms + protein + leukocyte count + lymphocytes % + ADA	High sensitivity and specificity	[63]
ADA + lymphocyte/neutrophil ratio	High sensitivity and specificity	[64]

ADA: adenosine deaminase; IFN: interferon; NAAT: nucleic acid amplification test.

and PCR were tested in the same samples for the diagnosis of microbiologically and/or histologically confirmed pleural TB. The sensitivities of ADA activity, IFN-γ levels and PCR were 88, 86 and 74%, respectively, and the specificities were 86, 97 and 90%, respectively. The combination of PCR, IFN-γ and ADA activity allowed an increase of sensitivity and specificity compared with individual methods in isolation. However, in other studies using different tests in the same samples, in which ADA activity had a higher accuracy, no other test significantly added sensitivity to the ADA [20, 65].

In a logistic regression model, a combination of duration of disease, protein levels, total leukocyte count, percentage of lymphocytes and ADA activity measurement was >95% sensitive and specific for the diagnosis of TB in a high prevalence setting [63]. The first four variables added specificity to ADA activity, without loss of sensitivity. The lymphocyte/neutrophil ratio has also added to ADA specificity in another study of 472 pleural effusions [64].

More models, which take into account clinical features and simple, inexpensive and rapid diagnostic tests, can be developed with the use of modelling tools. These models are highly relevant to clinicians practising in poor resource settings. Even when ADA is unavailable, use of demographic and clinical information in combination with a cell differential count, in high incidence settings, can yield a diagnosis of TB with a high predictive value.

EMERGING TECHNOLOGIES THAT NEED TO BE EVALUATED FOR PLEURAL TB

In the past few years, several new TB diagnostics have been developed. These include tests such as loop-mediated isothermal amplification, detection of LAM (a TB antigen) using ELISA, phage-based assays and rapid culture systems [66, 67]. To date, none of these have been adequately evaluated for TB pleuritis.

In addition to laboratory tests, biological processes found in nature are being used to inspire the development of new technologies. Artificial intelligence represents an attempt to simulate the human brain in resolving complex questions by breaking them down into small problems. Neural network systems have been used to extract relevant information from complex databases, categorise them into groups with similar characteristics and organise them into distinct patterns [68]. The neural network system can help in identifying patients' variables that may not make biomedical sense but could be relevant for the diagnosis of diseases. It can also detect errors in databases. This tool has been useful in predicting pulmonary TB in patients with negative sputum smears [69, 70]. The tool can also easily generate scoring models based on clinical and laboratory data. Research is needed to evaluate the usefulness of neural network systems for the diagnosis of pleural TB.

FUTURE DIRECTIONS

Table 3 presents some suggested research directions. Diagnostic studies on pleural TB are particularly needed in specific populations, including individuals with HIV infection, children and other high-risk populations where traditional diagnostic tests have worse yield. In addition, commercially available tests for diagnosis of pulmonary TB should be validated for diagnosis of pleural TB. Better tests are needed, and/or available tests, such as NAAT or serologic tests that have good specificity, need to be modified to improve sensitivity.

Multidrug-resistant and extensively drug-resistant TB are spreading worldwide and have become a special issue for TB control. There are no reports on the drug-resistance profile of strains isolated from pleural specimens, but given the trend of ever-increasing resistance, development of new techniques, such as NAATs, to identify drug-resistant strains causing pleural TB will be important.

Efforts are also needed to improve the quality and reporting of TB diagnostic studies, according to the Standards for Reporting of Diagnostic Accuracy recommendations [71]. The main limitation of many published studies is the absence of a well-defined reference standard (culture and histopathological evidence of TB), leading to test performance results that may be biased. In addition to the lack of a definitive gold standard, diagnostic studies are plagued by methodological and design flaws that compromise validity, such as lack of a consecutive or

TABLE 3 Recommendations for future research

Test or biomarker	Research needs and recommendations
Nonspecific inflammatory and immune response markers	Commercial kits for ADA need to be validated in large studies with well-defined reference standards. The roles of cytokines and biological markers other than IFN-γ need to be established, which may require genomic or proteomic approaches.
Specific markers of immune response	Potentially should provide high specificity; further studies, particularly in high-incidence settings, required.
T-cell response	Improvements needed in specificity and sensitivity of IGRAs, and DNA extraction methods. Antigens other than ESAT-6 and CFP-10 need to be tested.
B-cell response (antibodies)	Antibodies against glycolipid antigens need to be developed and tested. Antibodies to protein antigens need sensitivity improvement, and more immunogenic antigens need to be found.
Detection of *Mycobacterium tuberculosis* nucleic acid by amplification tests	Potentially should provide high specificity; need sensitivity improvement. More commercial tests need to be developed. Existing kits need to be validated in pleural fluid. Potential to provide drug sensitivity profile.
Combinations of markers	More predictive models need to be developed using different clinical and laboratory data, with aid of informatics; artificial intelligence.

ADA: adenosine deaminase; IFN: interferon; IGRA: IFN-γ release assay; ESAT: early-secreted antigenic target; CFP: culture filtrate protein.

random patient-sampling method, use of a case–control design (where severe cases are often compared with healthy controls) and lack of blinding [72–74]. Statistical analysis of results should receive special attention in future studies [21, 75]. Sensitivities and specificities are proportions, and their confidence intervals must be calculated and reported [75]. When using statistical tests for comparison of sensitivities, only patients with the disease should be compared, while for specificity, only patients without the disease should be compared [21].

CONCLUSION

Diagnosis of pleural TB remains a challenge, as summarised in table 1. Among nonconventional tests, ADA and IFN-γ have the best sensitivity and specificity, but they are biomarkers of the inflammatory process in the pleural space and do not confirm the aetiologic agent. NAATs and serology are promising but need further development to improve sensitivity. Limited evidence is available for other novel tests and biomarkers.

Combinations of tests seem to perform better than any single test, especially combinations that include adenosine deaminase, but only a few studies have evaluated scoring systems (table 2). Further work is necessary to identify the best (and simplest) combination that will be most useful in clinical practice.

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