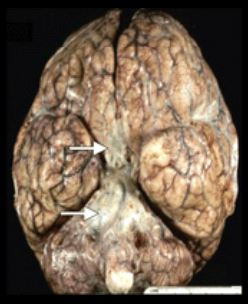
Extrapulmonary TB (EPTB)

Reference standards for diagnostic studies





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Struggling against EPTB for millennia

- Man & TB intertwined for >2 million years
- Possible Bone TB – diagnosed by anatomical appearance and MTB PCR

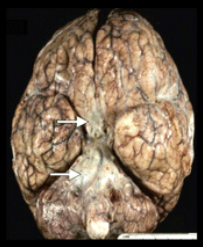
Atlit-Yam, Haifa, Israel, 2008

Which PCR was used?
How sure are we that it was really TB?

Need to label it “possible TB”

Sites for EPTB

Almost any part of body!
Common important diagnostic groups:
1) Pleural TB
2) CNS TB
3) Abdominal TB
4) Lymph node TB
5)Bone and Jt TB



Performance of TB Culture

- Variable mostly poor for all forms of EPTB
- Pleural TB – 24-58%
  Trajman A, Pai M & Dheda K *ERJ* 2008
- TB meningitis – 52-87% (87% from 4 lumbar punctures)
  Kennedy DH, Fallon RJ *JAMA* 1979
- Abdominal TB – ascitic fluid up to 83% (when 1 litre ascitic fluid tapped and centrifuged)
  and tissue as low as 7%
  Singh MM, Bhargava AN, Jain KPK *NEJM* 1969
  Khan R, Abid S, Jafri W, Abbas Z *World J Gastroenterology* 2006
- Lymph node TB ('Scrofula') – 62% FNA, 71% biopsies
  Polesky A, Grove W, Bhatia G *Medicine (Baltimore)* 2005

e the op

All use one/combination of the

1) Tissue biopsy/histopatholog
2) Case definitions/scoring systems – utilise combinations of clin radiology
3) Response to anti-TB treatment
4) Combinations of biomarkers and diagnostic tests

- Wide variations across studies
- Wide variation in performance
- Influenced by disease prevalence

therefore not applicable across settings

- Invasive
- Often unavailable
- Expensive
- False negatives in 15-20% cases
- Other diseases causes granulomatous inflammation
- AFBs maybe represent NTMs

- TB treatment effective for bacterial disease
- No response does not always mean no disease
- Some alternative diagnoses respond without Rx e.g. Viral meningitis

- Dependent on non-redundancy of test performance
- Expensive to perform numerous tests
- Inclusion bias if including new test

Diagnostic categories for EPTB

1) Definite TB – *Mtb.* Culture positive ± histopathological evidence ± other test (e.g. NAAT for TBM with high specificity)
2) Probable TB – Culture negative and fulfills case definitions/ high on scoring systems ± response to anti-TB Rx
3) Possible TB – lowering on scoring systems/not fulfilling case definition but responding to TB Rx
4) Indeterminate e.g. lost to follow-up, NB report
5) NON TB – alternative diagnosis proven, no anti-TB Rx, no TB at follow-up (usually >2 months)

Pleural TB studies

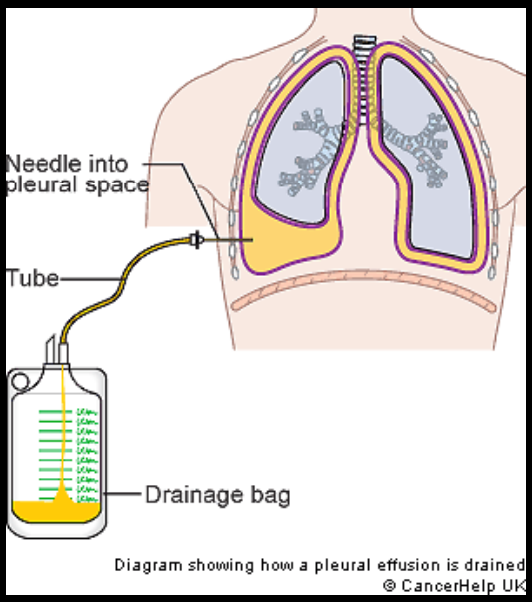


Diagram showing how a pleural effusion is drained
© CancerHelp UK

Culture/histopathological reference

Utility of quantitative T-cell responses *versus* unstimulated interferon-γ for the diagnosis of pleural tuberculosis

K. Dheda*,#,¶, R.N. van Zyl-Smit*, L.A. Sechi+, M. Badri*, R. Meldau*, S. Meldau*, G. Symons*, P.L. Semple*, A. Maredza*, R. Dawson*, H. Wainwright§, A. Whitelaw/, Y. Vallie*, P. Raubenheimer*, E.D. Bateman* and A. Zumla¶

The reference standard used for diagnosis of TB was culture positivity for *Mycobacterium tuberculosis* (using pleural fluid or tissue) and/or histology suggestive of TB (caseous necrosis with acid-fast bacilli, with or without granuloma formation). Patients were thus characterised as 1) definite TB (meeting the reference standard), 2) non-TB (no microbiological or histological evidence for TB, alternative diagnosis made, not treated for TB and did not develop TB over 6-month follow-up), and 3) probable TB (empirical anti-TB treatment but not meeting the criteria for definite TB). The definite and non-TB groups were used for sensitivity and specificity calculations. All assays were performed by an experienced laboratory technician who was blinded to patient and clinical details.

→ Report reference std

→ Describe diagnostic categories

→ Describe groups used for accuracy measures

Scoring systems/combination references

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.

Trajman A, Pai M, Dheda K & Menzies D *ERJ* 2008

Specific example

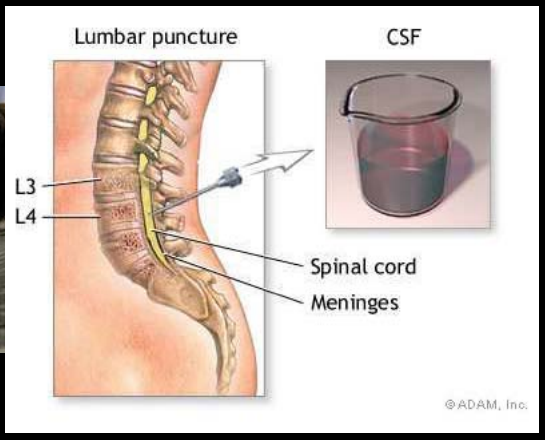
Porcell and Vives *Med Sci Monit* 2003 (Spain)		Sensitivity	Specificity
Age<35years (2 points) Temperature ≥ 37.8°C (2 points) Pleural fluid rbc count < 5 x 10^9/l (1 points)	5 pts	95%	94%

Villegas MV, Labrada LA & Saravia NG *Chest* 2000			
ADA, unstimulated IFN-ɣ, NAAT alone or in combination		- sensitivity same	Specificity ↑

Latent class analysis

- Statistical method to compensate for imperfect “reference” tests
- A probabilistic model is assumed for the relationship between the new diagnostic test, one or more imperfect “reference” tests, and the unobserved, or latent, disease status
- Minimum of 3 “conditionally independent” tests
- Call the statistician!

TB meningitis studies and attempts to unify case definitions for diagnostic studies



Panel 1: Examples of tuberculousis case definitions used for adults, children, or both in the past 5 years

Adults only

Torok (2008)[17]
- Patients: HIV seropositive, ≥15 years of age
- Case definition includes definite and probable tuberculous meningitis cases
- Definite tuberculous meningitis: CSF smear positive for AFB and/or culture positive for *Mycobacterium tuberculosis*
- Probable tuberculous meningitis: clinically suspected tuberculous meningitis plus one or more of the following four criteria:
  - i) CXR consistent with pulmonary tuberculosis
  - ii) Other specimens (eg, sputum, lymph node, gastric washings) positive for AFB
  - iii) Evidence of extrapulmonary tuberculosis
  - iv) CT or MRI evidence of tuberculous meningitis
- Patients were excluded if there was microbiological evidence of another CNS infection.

Kalita (2007)[9]
- Patients: HIV seronegative, ≥13 years of age
- Case definition includes definite and suggestive tuberculous meningitis cases.
  - A) Clinical criteria: meningitic symptoms including fever, headache, and vomiting for 2 or more weeks
  - B) Supportive criteria:
    - i) CSF cells ≥20/μL with predominant lymphocytes, protein ≥2 g/L
    - ii) CT scan evidence of exudates, infarctions, hydrocephalus, and tuberculoma in various combinations
    - iii) Evidence of extra-CNS tuberculosis
    - iv) Response to antituberculosis therapy
  - C) Exclusion criteria: malaria, septic, fungal, and carcinomatous meningitides
- Definite tuberculous meningitis: A and C plus positive PCR for *M tuberculosis* or IgM ELISA , or AFB in CSF smear or culture
- Suggestive tuberculous meningitis: A, C, and three or more of B

Thwaites (2004)[10]
- Patients: HIV seropositive and negative, >14 years of age
- Case definition includes definite, probable, and possible tuberculous meningitis cases
- Definite tuberculous meningitis: clinical meningitis (nuchal rigidity and abnormal CSF parameters) and AFB in the CSF
- Probable tuberculous meningitis: clinical meningitis and one or more of the following:
  - i) Suspected active pulmonary tuberculosis on the basis of CXR
  - ii) AFB found in any sample other than from the CSF
  - iii) Clinical evidence of other extrapulmonary tuberculosis
- Possible tuberculous meningitis: clinical meningitis and four or more of the following:
  - i) History of tuberculousis
  - ii) Predominance of lymphocytes in the CSF
  - iii) Illness of more than 5 days in duration
  - iv) CSF to blood glucose ratio of less than 0·5
  - v) Altered consciousness
  - vi) Yellow CSF
  - vii) Focal neurological signs
- Patients were subsequently reclassified as having definite tuberculous meningitis if AFB were seen in or *M tuberculosis* was cultured from the CSF, and as not having tuberculous meningitis if another diagnosis was confirmed by microbiological or histopathological assessment

Adults and children

Nagesh Babu (2008)[11]
- Case definition includes definite and presumptive tuberculous meningitis cases
  - A) Clinical criteria: fever, headache, meningeal signs, and other clinical presentations of meningitis lasting for more than 2 weeks.
  - B) CSF criteria: typical features including pleocytosis (> 20 cells/μL), lymphocytes >60%, protein >1 g/L, and CSF: blood glucose ratio of less than 0·6
  - C) Supportive criteria:
    - i) Isolation of *M tuberculosis* from body secretion other than CSF in smear or culture
    - ii) CXR findings of pulmonary tuberculosis (reticulonodular pattern in upper lobes with or without cavitary lesions)
    - iii) Hydrocephalous from brain CT scan
  - D) Negative bacterial and fungal cultures and negative India ink
- Definite tuberculous meningitis diagnostic criteria not stated
- Diagnosis of presumptive tuberculous meningitis requires A, B, one or more of C, and D to be fulfilled

Rafi (2007)[13]
- Patients: HIV seropositive and negative
- Case definition includes culture-confirmed and clinical tuberculous meningitis cases
- Diagnosis of clinical tuberculous meningitis requires A, B, and C:
  - A) Clinical findings: headache, fever, and vomiting for more than 3 weeks
  - B) CSF findings: pleocytosis and high protein concentration
  - C) Neuroimaging findings: the presence of a basal exudate with or without hydrocephalus

(Continues on next page)

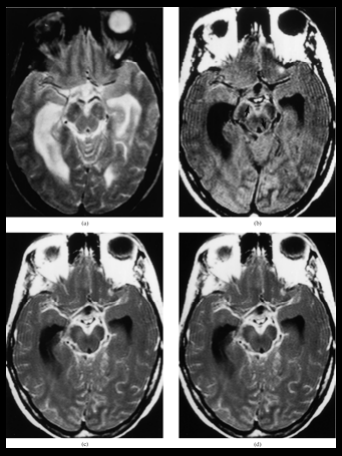
Marais S et al *Lancet* 2010

Multiple case definitions for TBM - 14 different ones referenced!

All involve combinations of:
1) Clinical
2) CSF findings ±
3) Radiology ±
4) TB Rx response

A uniformed case-definition

- Absence of standardisation in TBM diagnostic categorisation and clinical case definition makes comparison of studies difficult
- May 2009 – Cape Town - TBM workshop
- Develop consensus case-definition for future studies and evaluation of new tests



Panel 2: **Consensus tuberculous meningitis diagnosis**

Clinical entry criteria

- Symptoms and signs of meningitis including one or more of the following: headache, irritability, vomiting, fever, neck stiffness, convulsions, focal neurological deficits, altered consciousness, or lethargy.

Tuberculous meningitis classification

Definite tuberculous meningitis

- Patients should fulfill criterion A or B:
  - A) Clinical entry criteria plus one or more of the following: acid-fast bacilli seen in the CSF; *Mycobacterium tuberculosis* cultured from the CSF; or a CSF positive commercial nucleic acid amplification test.
  - B) Acid-fast bacilli seen in the context of histological changes consistent with tuberculosis in the brain or spinal cord with suggestive symptoms or signs and CSF changes, or visible meningitis (on autopsy).

Probable tuberculous meningitis

- Clinical entry criteria plus a total diagnostic score of 10 or more points (when cerebral imaging is not available) or 12 or more points (when cerebral imaging is available) plus exclusion of alternative diagnoses. At least 2 points should either come from CSF or cerebral imaging criteria.

Possible tuberculous meningitis

- Clinical entry criteria plus a total diagnostic score of 6–9 points (when cerebral imaging is not available) or 6–11 points (when cerebral imaging is available) plus exclusion of alternative diagnoses. Possible tuberculosis cannot be diagnosed or excluded without doing a lumbar puncture or cerebral imaging.

Not tuberculous meningitis

- Alternative diagnosis established, without a definitive diagnosis of tuberculous meningitis or other convincing signs of dual disease.

CSF=cerebrospinal fluid.

Marais S et al *Lancet* 2010

Clinical entry criteria – patients must have symptoms of meningitis

Definite TB:
1) CSF smear or culture positive or CSF PCR
2) AFB in context of histology with CSF changes

Probable TB and possible TB:
1) Clinical entry criteria + diagnostic score

Not TB:
1) Alternative diagnosis established

Removed TB Rx

	Diagnostic score
Clinical criteria	(Maximum category score=6)
Symptom duration of more than 5 days	4
Systemic symptoms suggestive of tuberculosis (one or more of the following): weight loss (or poor weight gain in children), night sweats, or persistent cough for more than 2 weeks	2
History of recent (within past year) close contact with an individual with pulmonary tuberculosis or a positive TST or IGRA (only in children <10 years of age)	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
Altered consciousness	1
CSF criteria	(Maximum category score=4)
Clear appearance	1
Cells 10–500 per µl	1
Lymphocytic predominance (>50%)	1
Protein concentration greater than 1 g/L	1
CSF to plasma glucose ratio of less than 50% or an absolute CSF glucose concentration less than 2·2mmol/L	1
Cerebral imaging criteria	(Maximum category score=6)
Hydrocephalus	1
Basal meningeal enhancement	2
Tuberculoma	2
Infarct	1
Pre-contrast basal hyperdensity	2
Evidence of tuberculosis elsewhere	(Maximum category score=4)
Chest radiograph suggestive of active tuberculosis: signs of tuberculosis=2; miliary tuberculosis=4	2/4
CT/ MRI/ ultrasound evidence for tuberculosis outside the CNS	2
AFB identified or *Mycobacterium tuberculosis* cultured from another source—ie, sputum, lymph node, gastric washing, urine, blood culture	4
Positive commercial *M tuberculosis* NAAT from extra-neural specimen	4
Exclusion of alternative diagnoses	
An alternative diagnosis must be confirmed microbiologically (by stain, culture, or NAAT when appropriate), serologically (eg, syphilis), or histopathologically (eg, lymphoma). The list of alternative diagnoses that should be considered, dependent upon age, immune status, and geographical region, include: pyogenic bacterial meningitis, cryptococcal meningitis, syphilitic meningitis, viral meningo-encephalitis, cerebral malaria, parasitic or eosinophilic meningitis (*Angiostrongylus cantonesis, Gnathostoma spinigerum*, toxocariasis, cysticercosis), cerebral toxoplasmosis and bacterial brain abscess (space-occupying lesion on cerebral imaging)and malignancy (eg, lymphoma)	

TST=tuberculin skin test. IGRA=interferon-gamma release assay. NAAT=nucleic acid amplification test. AFB=acid-fast bacilli. The individual points for each criterion (one, two, or four points) were determined by consensus and by considering their quantified diagnostic value as defined in studies.

Table: **Diagnostic criteria for classification of definite, probable, possible, and not tuberculous meningitis**

Marais S et al *Lancet* 2010

Case definition
Using weighted scoring:

1) Probable TB
   ≥ 10 (no radiology)
   ≥ 12 (radiology)
1) Possible TB
   6-9 (no radiology)
   6-12 (radiology)

TB Lymphadenitis

- Reflect similar issues as two previous examples
- Studies use culture positive or combinations of tests (e.g. AFB po suggestive or PCR p
- Complexities of resp
- Clinical scoring systems variable and complex

TB-Lymphadenitis on TB treatment
•7% persistence of enlarged nodes
•7% transient enlargement
•14% developed new nodes during therapy
•21% had residual adenopathy after therapy was complete
Daley P, Thomas S & Pai M *IJTLD* 2007

Conclusions

- No good reference standards for EPTB
- Various combination ‘reference’ standards used
- Reporting diagnostic categories and groups used for analysis imperative
- Consensus case definitions/disease categorisation will allow better comparison across studies