New approaches and the importance of clinical laboratory diagnosis in Extra-pulmonary Tuberculosis

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The 6th International & National Congress on Quality Improvement in clinical Laboratories Tehran- 2013

Definitions

Extrapulmonary tuberculosis (EPTB):

• Isolated occurrence of TB at body sites other than the lung

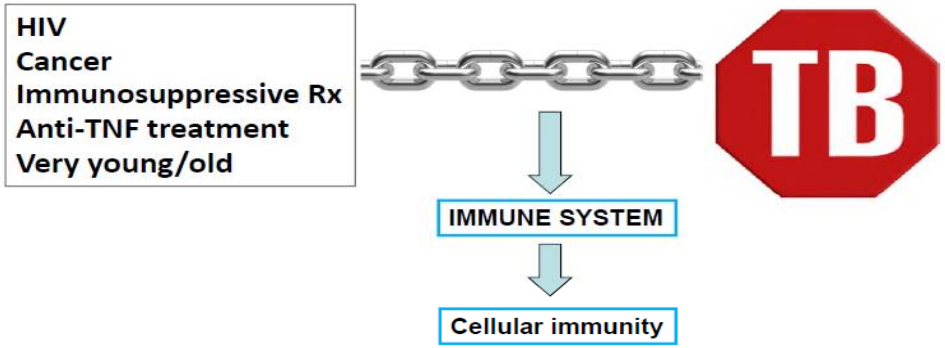
Extrapulmonary tuberculosis (EPTB) diagnosis:

• At least one specimen with confirmed M. tuberculosis or histological or strong clinical evidence consistent with active EPTB

• Case definition of an EPTB case with several sites affected depends on the site representing the most severe form of disease

• A patient with both PTB and EPTB should be classified as PTB

Pathogenesis



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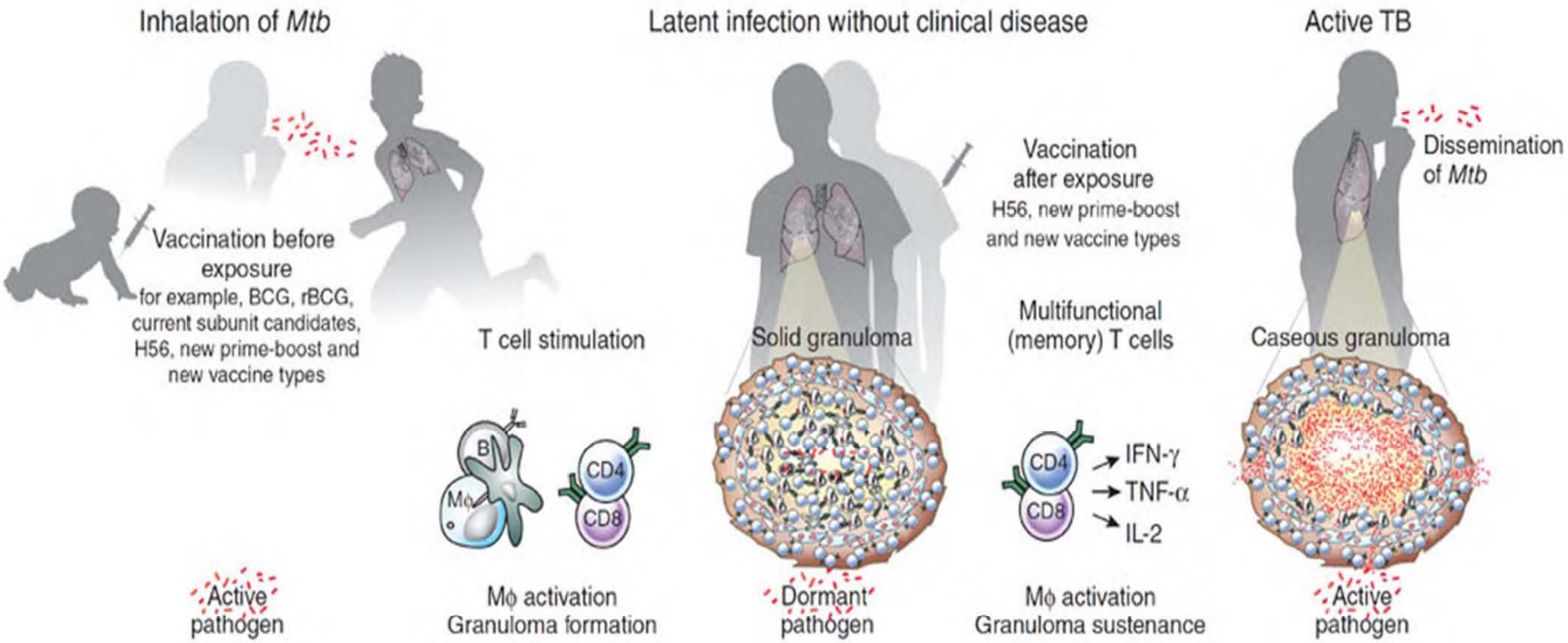


Manifestations of EPTB

- EPTB involves organs other than the lungs:
- Pleura
- Lymph nodes
- Abdomen
- Genitourinary
- Skin
- Joints and bones
- Meninges
- Other: skin, eye

Extra pulmonary Tuberculosis: a challenging diagnosis?

- The rapid and accurate diagnosis of TB in children and extrapulmonary TB in adults **continues to be a challenge**. ***Journal of Clinical Microbiology . 2012 : 50 (7).***

- **Diagnosis of EPTB remains especially challenging** since the number of bacilli present in tissues at sites of disease is often low and clinical specimens from deep-seated organs may be difficult to obtain. The Histology is time-consuming to undertake and establishing a diagnosis of TB with high specificity remains difficult. **Reliance on culture, often leads to considerable delays**, ***Expert Rev Anti Infect Ther. 2012 : 10(6).***

- Rates of smear-negative pulmonary and extrapulmonary TB are higher in HIV-infected compared with uninfected persons with TB. In these patients, severe immunosuppression and **delayed diagnosis are additional contributors of such excess mortality**. Similar issues exist for children in whom TB diagnosis is equally challenging due to a high proportion of sputum smear and culture negative disease. ***JID 2011:204.***

Extra pulmonary Tuberculosis: a challenging diagnosis?

- In 2009, CDC recommended the use of at least one molecular technique One of the molecular techniques most widely used for the detection of *M.* tuberculosis in respiratory samples is the commercial PCR kit Cobas Problems have been reported using this kit, **particularly with nonrespiratory samples, due to the presence of inhibitor with nonrespiratory samples, due to the presence of inhibitor enzymes and contamination.** ***JOURNAL OF CLINICAL MICROBIOLOGY,. 2011, 49(8).***

- **The number of bacteria in extrapulmonary specimens is often lower than** the number in pulmonary specimens. Furthermore, **collection of extrapulmonary material often requires invasive procedure**s, and **it is not easy to obtain additional samples.** ***JOURNAL OF CLINICAL MICROBIOLOGY, 2011,49(4).***

- A high index of suspicion is necessary to make an early diagnosis of EPTB, and quite often, **more than one procedure is necessary for the confirmation of** the diagnosis. In lower-income countries, **the lack of diagnostic infrastructure substantially aggravates the problem.** ***JOURNAL OF CLINICAL MICROBIOLOGY, 2011, 49(7).***

Better tools?

Various methods are employed for the Diagnosis of EPTB

- Smear Microscopy
- Culture Identification
- Histopathology
- Tuberculin skin test (TST)
- Serological histopathology
- Serological assays
- Interferon-gamma release assays (IGRAs)
- Nucleic acid amplification (NAA)

GeneXpert MTB/RIF

- The **Xpert MTB/RIF** is a cartridge-based, automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) and resistance to rifampicin (RIF).
- Professor David Alland at the University of Medicine and Dentistry of New Jersey .
- In December 2010, the World Health Organization (WHO) endorsed the Xpert MTB/RIF for use in TB endemic countries.
- The Xpert MTB/RIF detects DNA sequences specific for Mycobacterium tuberculosis and rifampicin resistance by polymerase chain reaction[It is based on the Cepheid GeneXpert system, a platform for rapid and simple-to-use nucleic acid amplification tests (NAAT).
- The Xpert® MTB/RIF purifies and concentrates Mycobacterium tuberculosis bacilli from sputum samples, isolates genomic material from the captured bacteria by sonication and subsequently amplifies the genomic DNA by PCR and identifies all the clinically relevant Rifampicin resistance inducing mutations in the RNA polymerase beta (rpoB) gene in the Mycobacterium tuberculosis genome in a real time format using fluorescent probes called molecular beacons.
- Results are obtained from unprocessed sputum samples in 90 minutes, with minimal biohazard and very little technical training required to operate

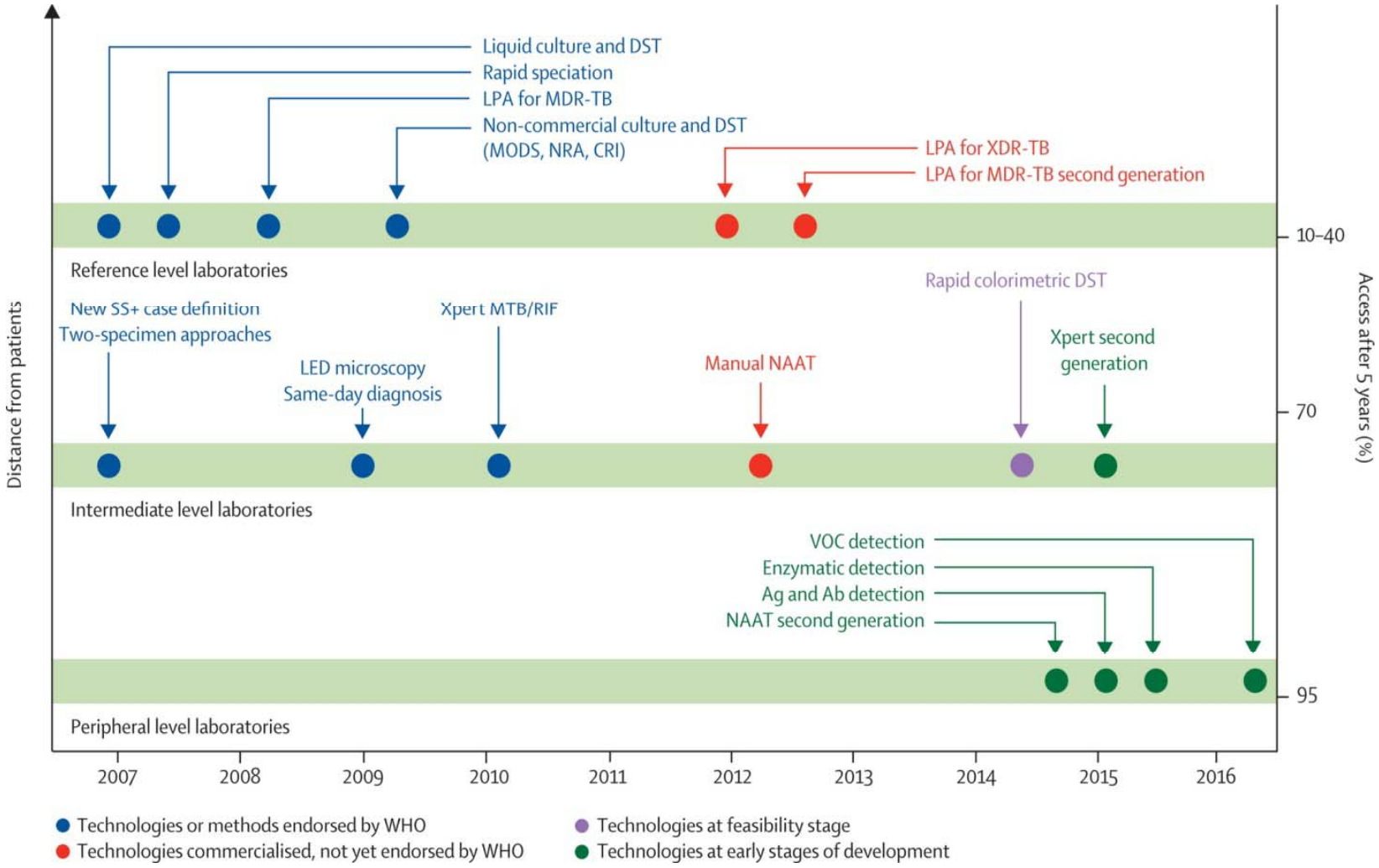
GeneXpert MTB/RIF

•The Xpert test has true diagnostic potential with good **sensitivity (86 to 100%)** for specimens such as synovial, pericardial, and peritoneal fluids; pus; and fineneedle aspirates and moderate **sensitivity (63 to 73%)** for tissues, lymph nodes, and pleural fluid but poor **sensitivity (29%)** in the case of CSF. ***JOURNAL OF CLINICAL MICROBIOLOGY, 2011, 49(7).***

•(**sensitivity:81.3%**)(**specificity: 99.8%**) ***Expert Rev Anti Infect Ther. 2012, 10(6).***

•Of the 521 specimens the **sensitivity** of the Xpert assay with tissue specimens was **69.0%** and **100% sensitivity** was found with the urine and stool specimens. ***JOURNAL OF CLINICAL MICROBIOLOGY, 2011, 49((4)***

The Xpert MTB/RIF assay, which enables simultaneous detection of *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance, was endorsed by WHO in December, 2010. ***The Lancet Infectious Diseases,2013, 13 (4).***



Terms and Conditions

Serodiagnostic utility of novel Mycobacterium tuberculosis polyproteins

- Two novel M. tuberculosis polyproteins, 38kD-ESAT6-CFP10 (38F) and Mtb8.4-MPT64-TB16.3-Mtb8 (64F), were expressed and the novel 38F-64F indirect ELISA assay used to analyze antibody responses to polyproteins in serum samples. The novel 38F-64F indirect ELISA had a sensitivity of 74.16% and specificity 90.36% with sera from extrapulmonary TB patients ***J Infect. 2013 Apr;66(4):366-75***.

- **Use of ESAT-6 and CFP-10 Antigens for Diagnosis of Extrapulmonary TB**. data show that a TB immunodiagnostic blood test containingESAT-6 and CFP-10 holds a strong potential for thediagnosis of active EP-TB and P-TB .JID 2001;183 (1 January)

Super-paramagnetic iron oxide nanoparticles for use in extrapulmonary tuberculosis diagnosis

- The limited sensitivity of serological tests for mycobacterial antigens has encouraged the development of a nanoparticle probe specific for the extrapulmonary TB. Innovative probe comprised of super-paramagnetic iron oxide (SPIO) nanoparticles conjugated with Mtb surface antibody (MtbsAb-nanoparticles) to provide ultrasensitive imaging of biomarkers involved in extrapulmonary Mtb infection When MtbsAb-nanoparticles were intravenously injected into mice bearing Mtb granulomas, the granulomatous site showed a 14-fold greater reduction in signal intensity enhancement on T(2) -weighted MR images compared with an opposing site that received PBS injection. **Mtb sAb-nanoparticles represent a new non-invasive technology for the diagnosis of extrapulmonary Mtb.** ***Clin Microbiol Infect. 2012 Jun;18(6):E149-57.***

Enzyme-linked immunospot assay (ELISPOT)

- Adaptive immunity is an important component to clearance of intracellular pathogens. The ability to detect and quantify these responses in humans is an important diagnostic tool. **The enzyme-linked immunospot assay (ELISPOT)** is gaining popularity for its ability to identify cellular immune responses against microbial antigens. **ELISPOT is not limited to the site of inflammation. It is versatile in its ability to assess for immune responses within peripheral blood, as well as sites of active involvement such as bronchoalveolar lavage, cerebral spinal fluid, and ascites.** Detection of immune responses against a single or multiple antigens is possible, as well as specific epitopes within microbial proteins. Dual color ELISPOT assays are available for detection of simultaneous expression of two cytokines. Recent applications for this technique include diagnosis of **extrapulmonary tuberculosis**, as well as investigation of the contribution of infectious antigens to autoimmune diseases. ***Vis Exp. 2010 Nov 23;(45).***

Multiplex real-time PCR assay based on SYBR Green I

- Some sites of extrapulmonary tuberculosis and focal complications of brucellosis are very difficult to differentiate clinically, radiologically, and even histopathologicallyConventional microbiological methods for the diagnosis of extrapulmonary tuberculosis and complicated brucellosis not only lack adequate sensitivity, they are also time consuming, which could lead to an unfavourable prognosis. The IS711, bcsp31 and omp2a genes were used for the identification of *Brucella* spp and **the IS6110, senX3-regX3 and cfp31 genes were targeted for the detection of the *M. tuberculosis* complex**. As a result of the different combinations of primers, nine different reactions were evaluated. A test was defined as positive only when the gene combinations were capable of co-amplifying both pathogens in a single reaction tube and showed distinguishable melting temperatures for each microorganism. According to the melting analysis, only three combinations of amplicons (senX3-regX3+bcsp31, senX3-regX3+IS711 and IS6110+IS711) were visible. Detection limits of senX3-regX3+bcsp31 and senX3-regX3+IS711 were of 2 and 3 genome equivalents for *M. tuberculosis* complex and *Brucella* while for IS6110+IS711 they were of 200 and 300 genome equivalents, respectively. **The three assays correctly identified all the samples, showing negative results for the control patients**. In conclusion, multiplex real time PCR assays based on the targets senX3-regX3+bcsp31 and senX3-regX3+IS711 using SYBR Green I are highly sensitive and reproducible. This may therefore be a practical approach for **the rapid differential diagnosis between extrapulmonary tuberculosis** and complicated brucellosis. **PLoS One. 2013; 8(3): e58353.**