Diagnosis of tuberculosis: Update on interferon-gamma release assays in clinical practice

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9.15 million of people suffer from tuberculosis worldwide

BUT

the RESERVOIR of infected people is by far more important

1/3 of the world population infected: ~2 billions of individuals

One new person is infected every second

8 – 10 millions of people are infected each year

Development of evidence-based policies on TB diagnosis is urgently needed for effective control of the global TB epidemic

Tuberculosis: natural evolution

Aerosol exposure

Primary infection

Latent infection

Reactivation

Active tuberculosis

Elimination of the bacteria

5%

95%

5%

5%

Re-infection

Active tuberculosis

Latent TB

Identification and treatment of subjects at risk to develop active TB

RECENT infections (< 2 yrs)

BEFORE starting immunosuppressive treatment

Subjects with persistent bacterial multiplication?

Active TB

Early treatment

to diminish morbidity and mortality

to diminish transmission

Importance of diagnostic
**Mycobacterium tuberculosis Infection**

- **Active TB:** 5-10%
  - Active infection: bacterial replication maintained at a subclinical level
  - Quiescent infection: controlled infection with persistence of a few NON REPLICATING bacteria
  - Infection eliminated with persistant sensitised T lymphocytes = acquired immunity

- **Latent TB:** 90-95%
  - Infection eliminated = innate immunity

**Initial LUNG infection**

- The bacteria reach the LYMPH NODES:
  - 8-9 days in circulation
  - 18-20 days

**Acquired immune response**

- **Naive T cells**
- **Effector T cells**

**Detection of this specific IFN-γ synthesis can be done by different techniques**

- ELISA
- ELISPOT
- QuantiFERON-TB Gold assay
- T-SPOT.TB

**Evolution of the test:**

- 2001 (USA): QuantiFERON-TB assay: PPD from Mycobacterium tuberculosis
- 2004 (USA): QFT-TB Gold: early secretory antigenic target-6 (ESAT-6) culture filtrate protein (CFP-10) antigens NOT encoded by the genome of BCG by the genome of most nontuberculous mycobacteria except: M. avian, M. leprae, M. lepraemurium, M. szulgai
- 2005 (Europe): QFT-TB Gold In-Tube (QFT-G IT): early secretory antigenic target-6 (ESAT-6) culture filtrate protein (CFP-10) TB7.7 peptide
QuantiFERON-TB Gold assay

Methodology

Mitogen: + control
Nil: - control
TB antigens

37°C 16-24 hrs

Plasma collection

POS: IFN-γ (TB-nil) ≥ 0.35 IU/ml

INDETERMINATE:
mitogen < 0.5 IU/ml
background response > 8.0 IU/ml

Advantages / TST

• Very high specificity
• does not boost responses
• does not require a 2nd clinical contact
• results theoretically available within 24 hrs

BUT...

• sensitivity? Difficult to evaluate...
• reproducibility over time?
• risk to develop active TB in patients with a positive results?
• requires lab equipment (incubation within 16 hrs – Elisa)
• requires blood sampling (3 mL)
• Expensive (25 E)

SENSITIVITY

• evaluation hampered by the lack of a gold standard for the diagnosis of LTBI
• test results are compared to those of the TST in high- and low-risk populations
    
    Active TB
    Contacts of active TB
    Low TB incidence countries
    No contact

• QFT-G does not discriminate between active and latent TB
• DIAGNOSIS of ACTIVE TB: QFT-G IT is NOT recommended:
    false negative results (low cellular immune responses) and
    false positive results (latent infection)

• DIAGNOSIS of LATENT TB:
    • The main advantage of QFT over TST is its ability to overcome false positive skin tests:
        - in BCG-vaccinated individuals
        - in patients who may be infected with non-TB mycobacteria
    
    Recommended use of IGRAs only to confirm a positive TST result in contacts with a low probability of acquired TB
    (Canada, UK, Germany)

    • Diagnostic value in immunocompromised patients incompletely assessed: numerous indeterminate results

    • Different studies suggest a lower sensitivity of QFT-G IT / TST for past infection:
        probably not suitable for the detection of latent TB before starting an immunosuppressive treatment
**QuantIFERON-TB Gold assay**

**SPECIFICITY**

Healthy « non-infected » subjects
- TST 13 mm – BCG – TB contact
- TST 15 mm – BCG – TB contact
- TST doubtfull – BCG – NO TB contact
- TST neg - NO TB contact

Old patients (≥ 70 yrs)
- TST 7.1 % +
- QFT + n=29 ; 34.1 %
- False + ?

- TST minimal – BCG – NO TB contact
- TST neg – NO TB contact

**ALTERNATIVE TESTS**

**T-SPOT TB**
- Detects the NUMBER of IFN-γ-secreting cells
- After stimulation with TB antigens: ESAT-6 and CFP-10
- Somewhat lower specificity / QFT (92 vs 97%)
- Slighty better sensitivity / QFT (88 vs 76 %)

- and fewer indeterminate results in the immunocompromised host

**HBHA IFN-γ-release assay**
- Hepatitis-Binding Hemagglutinin
- Methylated protein expressed at the surface of the members from the Mycobacterium tuberculosis complex
- Interferon-gamma release assay (IGRA) for the diagnosis of latent TB

**IGRA in response to HBHA**
- Sensitivity: 92.06 %
- Specificity: 93.88 %

*Hougardy JM et al. PLoS ONE, 2007*
DETECTION OF LATENT TB

nHBHA-IFN-γ release assay

NO INFLUENCE OF A PREVIOUS BCG VACCINATION (> 15 years)

CONTROLS

LTBI

Blood sample / TST conversion

< 2 yrs

> 2 yrs

The Quantiferon-TB Gold
(peptides of ESAT-6, CFP-10, TB 7.7)

QTF

nHBHA-IFN-γ

Importance of diagnostic

Latent TB

Active TB

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RECENT infections (< 2 yrs)

Early treatment
to diminish morbidity and mortality

BEFORE starting immunosuppressive treatment
to diminish transmission

Subjects with persistant bacterial multiplication?
Patients in end-stage renal diseases

R. Dessein et al.

Importance of diagnostic

Latent TB
- Identification and treatment of subjects at risk to develop active TB
  - RECENT infections (< 2 yrs)
  - BEFORE starting immunosuppressive treatment
- to diminish morbidity and mortality
- to diminish transmission

Active TB
- Early treatment
- Subjects with persistent bacterial multiplication?

Longitudinal follow-up during LATENCY

MOM52, BCG +

Corbière V et al, manuscript in preparation

Active TB
- Early treatment
- to diminish morbidity and mortality

Subjects with persistent bacterial multiplication?
HBHA-IGRA for the diagnosis of ACTIVE TB:

**LOCAL IGRA**

**PLEURAL FLUIDS**

Combining the IFN-γ response to 2 different mycobacterial antigens gives a higher sensitivity

Place S et al. Manuscript in preparation

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High local production of IFN-γ in response to HBHA

**BRONCHO-ALVEOLAR LAVAGES**

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HBHA-IGRA for the diagnosis of ACTIVE TB:

**LOCAL IGRA on PLEURAL fluids**

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**PERICARDIC – PERITONEAL – SYNOVIAL – CEREBROSPINAL FLUIDS**

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CONCLUSIONS

• IGRAs represent a major progress for the diagnosis of M. tuberculosis infection

• However, several progresses are still needed:
  • more data about the specificity and sensitivity
  • differential diagnosis between active and latent TB
  • marker of the risk to develop active TB
  • lower price

• Today, IGRAs represent an useful adjunct to the TST.
• Their result should always be interpreted within the clinical context

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