biomnis
SPECIALISED MEDICAL PATHOLOGY
Focus
issue 05

Mycobacterial infection diagnosis

Epidemiology

Tuberculosis worldwide

More than one third of the world's population, i.e. 2 billion people, are carriers of TB. Tuberculosis is the primary cause of infectious mortality in the world: 8 million people develop a tuberculosis disease every year and 2 million die from it. The AIDS epidemic has contributed to the development of the disease. In addition, the emergence of multi-resistant bacilli to antibiotics (Rifampin and Isoniazide) is being observed.

Tuberculosis in France

Its incidence is continuing to fall in industrialised countries. In France, it is less than 10 cases per 100,000 inhabitants (5 for the population born in France) and multi-resistance is approximately 1 %.

Mycobacterial infections

These include tuberculosis caused by *tuberculosis* group mycobacteria and "atypical" non-tuberculosis mycobacteria. More than 100 species of the latter are described: they are opportunistic, non-contagious ubiquitous micro-organisms found in water or soil, causing pulmonary infections, adenitis, skin infections and systemic forms.
These infections may be treated by means of surgery or medication: Clarithromycin + Ethambutol + Rifabutin.

Pathological mycobacterial diagnosis

Direct examination

This is performed after decontamination, concentration and fluidification if required. The first-line staining used is with auramine followed by Ziehl Neelsen for confirmation as it is more specific. This test is of low sensitivity (< 50 %) and non-specific but it makes it possible to indicate the presence or absence of acid and alcohol-fast bacilli and take rapid patient isolation and treatment measures.

La culture

This is the reference technique as it has the highest sensitivity, essential for identification and performing antibiotic susceptibility testing. It is performed on Löwenstein-Jensen and Coletsos solid media or, preferentially, on Middlebrook liquid medium and agar medium (liquid medium makes it possible to reduce the onset of growth by approximately 10 days with respect to solid media). Complementarity between solid and liquid media is recommended as some mycobacteria grow better in solid medium; others on the other hand grow better in liquid medium.

Identification

- The conventional biochemical method is long, tedious and subjective.
- Accuprobe® probes: tuberculosis complex, avian complex, M. kansasii, M. gordonae enabling a rapid response in 1 day but only 4 probes have been developed to date.
- Innogenetics Inn-Lipa® and Hain Mycobacteria Genotype® identify around fifteen species by means of PCR followed by molecular hybridisation.
- Hain MTBC Genotype® identifies within the tuberculosis complex (TB, bovis, africanum, microti, BCG).
- Sequencing enables exhaustive identification but involves databases.
- Molecular typing (MIRU-VNTR, spoligotyping, RFLP) is used to compare the identity of several strains.

Antibiotic susceptibility testing

- Tube proportion method on Lowenstein-Jensen medium: reference method still used in Pitié-Salpétrière Reference

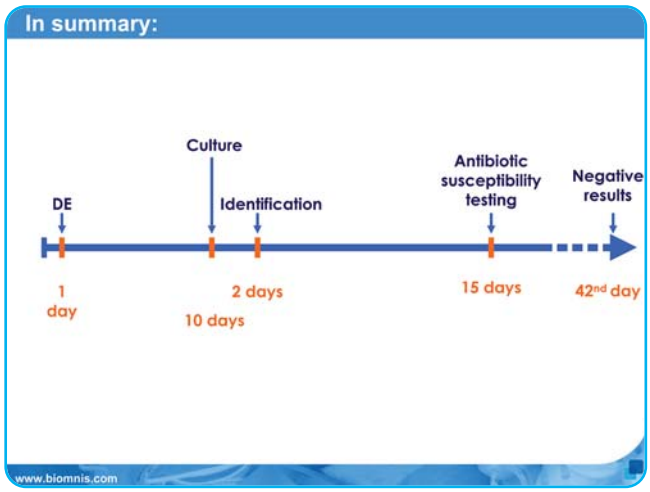
Centre with response in 28 days.

- MGIT Bactec® technique: response in 10 days for TB.
- Biomérieux BactAlert® for TB; marketing has been discontinued to date.
- E Test® (strips), Sensititre® (microplates) for rapid-growth atypical mycobacteria.

Rifampin and INH-resistance gene screening

Performed in emergency situations with suspected cases of multi-resistant TB. It is completed in 36 hours using positive cultures and positive direct examination samples.

- Rifampin: mutation of the rpoB gene is responsible for 96 % of resistances.
- INH (katG and INH A gene).



In summary: if culture is negative, the results are returned at 6 weeks; it is advised to wait a further 2 weeks as a precaution in the case of a strong suspicion.

Mycobacterial DNA and RNA screening

The two complexes that can be used are tuberculosis and avium. Real-time PCR and NASBA make it possible to return a result in less than 2 days. The sensitivity is low: 25 to 75 % when the direct examination is negative. The specificity is imperfect. The French schedule of medical procedures reserves testing to CSF and tissue with a negative direct examination. In any case, they must to be performed in addition to culture.

Interferon γ production blood test

Intradermal reactions (IDR) are difficult to implement and interpret. In addition, their specificity is low. For this reason, blood tests not overlapping with BCG have been developed. The French Higher Health Authority has selected them subject to the following conditions:

- Investigation of a case, at least 3 months after the presumed contact.
- Recruitment and monitoring of healthcare professionals.
- Diagnostic aid of extra-pulmonary forms of tuberculosis disease.
- Before starting an anti-TNFα treatment.

The **QuantiFERON-TB IT** test studies the ability of the patient's lymphocytes to secrete interferon γ after stimulation by ESAT-6, CFT-10 and TB7.7 proteins in TB (absent from BCG).

A negative antigen-free control and a positive control with mitogen validate the result.

Sampling is performed on 3 specific tubes; they must be shaken vigorously before incubating them for 24 hours at 37 °C, and centrifuging them without settling before shipping at 4 °C to the testing laboratory. Interferon γ is quantified using the ELISA technique.