

TUBERCULOSIS: NEW DEVELOPMENTS AND PERSPECTIVES

“The history of tuberculosis has been one of scientific, medical and political failure.”

According to the Global TB Control Report released one year later by the World Health Organization (WHO), the good news is that “the worldwide TB epidemic has leveled off for the first time since the disease was declared a public health emergency in 1993.” The bad news is that “at the current rate of progress, the 1990 prevalence and mortality rates will not be halved worldwide by 2015.” TB is the only disease ever declared a global emergency by the WHO.

Paradoxically, although we count on effective – and proven cost-effective interventions for its control, TB continues to cause great mortality and suffering, especially in poor and less-developed countries. Its association with the HIV/AIDS pandemic forms a lethal combination. In addition, multidrug resistant (MDR) TB and extensively drug resistant (XDR) TB – with further resistance to key second-line drugs and virtually incurable – severely complicate the management and control of the disease worldwide.

As repeatedly stated, one third of the world’s population is latently infected with *Mycobacterium tuberculosis* and 10 % of these people will develop active disease at some point in their life.

In the late ’70s - 80s, it was thought that TB could be eradicated from most developed and industrialized countries. TB was already regarded as a disease from the past and started to be neglected by medical doctors, scientists and agencies in charge of its control. However, this never became a reality, mainly due to the appearance of antibiotic resistance, and therefore, TB continues to be the big killer it was in the preantibiotic era. This unexpected reemergence of TB in the ’90s served not only to strengthen control measures but also to fuel research on TB.

Substantial scientific advances were made in knowledge about the agent and the disease in that decade. First, the complete genome sequence of the tubercle bacillus was decoded. Second, molecular epidemiology explained some mechanisms of TB transmission. Third, cellular mechanisms involved in *M. tuberculosis* resistance to several drugs were discovered. Fourth, new tools for speeding TB diagnosis and assessing drug susceptibility were worked out while old methods were rediscovered and/or reformulated. Lastly, research on drug and vaccine development exploded.

Bacillus and disease under the light of molecular epidemiology

In less than two decades, molecular epidemiology has changed our view of TB transmission dynamics, challenging traditional dogmas and answering unsolved epidemiological questions. Nowadays, molecular epidemiology is embedded within almost every aspect of TB research, laboratory diagnosis, clinical management, and control interventions.

The considerable amount of information gathered in national and international *M. tuberculosis* genotype databases throughout the world enabled the analysis of global TB dissemination and promoted phylogeographical studies that, in turn, incorporated more sophisticated and accurate markers of *M. tuberculosis* evolution.

A common conviction of previous times was that the genome of the tubercle bacillus was extremely stable and homogeneous. Phenotypical discrimination between strains – and even between species within the *M. tuberculosis* complex – was limited to phage typing and comparison of drug resistance patterns, which were the only tools available for differentiation. Molecular epidemiology discovered the existence of wide *M. tuberculosis* polymorphisms in population-based studies.

Molecular epidemiology tools also enabled the identification, description and differentiation of rare species within the *M. tuberculosis* complex and non-tuberculous mycobacteria. These species had previously been overlooked, mainly because they were difficult to distinguish by conventional biochemical tests.

In turn, differentiation to the species level by spoligotyping turned out to have practical implications on medical management and epidemiology. More recently, basic studies on genomics have been applied for the design of a clinical test – which is already available – for the rapid identification and differentiation of *M. tuberculosis* complex species, including BCG strains, in clinical isolates.

DNA strain typing is also a powerful tool for quality control of culture in diagnostic laboratories.

Other challenging issues raised by molecular epidemiology studies are related to reinfection and multiple infection, loss of strain fitness associated with drug resistance, differential virulence), tissue or organ affinity, vaccine development and protection. Unfortunately, this stimulating prospect poses a practical problem for laboratories in medium- and low-resource countries. The procedure is still too labor demanding. Thus, as long as science continues to advance, the scientific gap between industrialized and developing countries will widen.

New perspectives in diagnosis

In spite of the impressive advances made in the field with the existing tools, the ideal method for strain typing has not yet been achieved.

It is now 125 years since the tubercle bacillus was described by Robert Koch. Disappointingly, the diagnosis of the disease still relies on the same microscopy technique based on the specific Ziehl-Neelsen staining of the bacillus, which was already available soon after that fundamental discovery. Much more progress needs to be made in obtaining better and faster diagnostic methods. Indeed, in high-burden resource-poor countries, where TB is a major public health problem, the diagnosis of active disease is mainly performed by direct microscopic examination of sputum-smear samples. This technique, although simple and inexpensive, lacks sensitivity in comparison to *M. tuberculosis* culture. Several modifications – mainly based on concentration and centrifugation techniques – have been proposed to improve the sensitivity of sputum-smear microscopy, with varying results.

No other major improvement has been obtained in the classical staining method based on the Ziehl-Neelsen technique developed many years ago.

Fluorescent microscopy proved to be faster and more sensitive than microscopy based on Ziehl-Neelsen staining, and is the standard diagnostic method in high-income countries. It has the additional advantage of demanding less effort from the laboratorist, thus reducing fatigue and human error. As for low-income countries, the eventual introduction of fluorescent microscopy should be evaluated carefully because it requires a more expensive microscope and a more complex technique.

Cultivation of *M. tuberculosis* is the gold standard for the diagnosis of active TB in the laboratory. This has been traditionally performed in egg- or agar-based solid media. Although slow and time-consuming, it is relatively simple to perform and rather inexpensive in most settings. Newer alternative methods based on liquid culture media and giving faster results – such as the BACTEC radiometric method, have proven useful, especially in medium- and high-income countries. It is now standard recommendation that the combination of a solid and a liquid culture medium gives the best sensitivity in recovering mycobacteria in primary culture.

Immunological diagnosis.

Until recently, the tuberculin skin test was the only available test for the diagnosis of latent TB infection. Based on the detection of delayed-type hypersensitivity to purified protein derivative (PPD) obtained from *M. tuberculosis*, it measures the size of the induration produced after intra-cutaneous inoculation of a standardized dose. Since PPD is actually a raw mixture of several

antigens shared by *M. tuberculosis*, *M. bovis* BCG and several non-tuberculous mycobacteria, a positive tuberculin skin test result could be due to latent TB infection, previous BCG vaccination, or previous exposure to NTM. Additionally, it has various disadvantages, such as variability in the interpretation by different readers, the need of some experience to correctly interpret the result, and the requirement for the patient to return after 48-72 hours for test reading.

In order to overcome these disadvantages, immune-based blood tests have been recently introduced to detect latent TB infection. Interferon- γ (IFN- γ) assays measure the amount of IFN- γ produced or the actual number of IFN- γ -producing T lymphocytes in response to specific antigens. Both approaches are based on the fact that T cells sensitized with tuberculous antigens will produce IFN- γ when they are re-exposed *ex vivo* to mycobacterial antigens; a high amount of IFN- γ production is then presumed to correlate with TB infection.

The current IFN- γ assays use more specific antigens to *M. tuberculosis* than PPD. However, the absence of a real gold standard for the diagnosis of latent TB infection prevents a more definitive conclusion. Further studies comparing these two assays are needed, especially in immunosuppressed patients.

It should be remarked that TB diagnosis in endemic countries has so far gained little benefit from scientific progress. There is an urgent demand for a field-friendly test, ideally, a point-of-care one able to diagnose the disease on the spot in order to avoid delays in diagnosis, thus, preventing further transmission and reducing complications. This type of test is particularly useful when patients do not return for care and would greatly benefit people in settings such as prisons, homeless shelters, and clinics for migrant workers who have no ready access to, or do not seek, public health service assistance. The ideal diagnostics for TB should be available in one hour and should require no electricity, refrigeration and highly trained personnel.

The problem of drug resistance detection

Traditionally, drug resistance in TB was assessed by culturing *M. tuberculosis* on solid media in the presence of antibiotics and measuring growth by enumeration of colonies. This method, although simple to perform and rather inexpensive, is quite slow and laborious, requiring several weeks to give the final results. Many alternative approaches and methods have been proposed.

The most important consideration before they can be implemented in the routine diagnostic laboratory is that they are better and faster than the currently available methods. Several molecular tools have also been developed and proposed as rapid methods to detect drug resistance. They search for genetic determinants of

resistance rather than for the resistance phenotype, and involve molecular nucleic acid amplification by PCR and detection of amplified products for specific mutations correlating with drug resistance. Molecular methods have several advantages over culture-based techniques: shorter turnaround time, no need for growth of the organism, the possibility for direct application in clinical samples, less biohazard risks. However, not all molecular mechanisms of drug resistance are known.

On drug development

Associated with the problem of drug resistance is the search for new anti-tuberculosis drugs. Almost no new anti-tuberculosis drug classes have been developed over the last 40 years. In fact, the leading pharmaceutical industries lost interest in the development of anti-tuberculosis drugs. This lack of investment became evident when the HIV/AIDS pandemic emerged, soon followed by MDR-TB and the unavoidable interactions between anti-tuberculosis and anti-retroviral drugs. In view of this, interest in the discovery of new drugs against TB was awakened towards the end of the millennium. Many candidate compounds have been considered in the last decade, but very few of them have entered into further evaluations. These potentially useful anti-tuberculosis drugs are currently in different stages of the evaluation pipeline.

The Global Alliance for TB Drug Development was established in 2000 to promote the development of new anti-tuberculosis compounds. Its goal is to bring a new anti-tuberculosis drug onto the market by 2020.

The most advanced program on new drugs is examining bedaquiline and delamanile. Other promising drugs are in the pre-clinical phase of evaluation. The availability of such a spectrum of new drug candidates offers great promise but also entails a great challenge. The role played by each drug must be explored within the frame of a multidrug treatment regimen. In the immediate future, a complete series of clinical trials will be needed to find the optimal treatment scheme of ultra-short duration, i.e. 2 months or even shorter. Obviously, many more efforts and funding are required to reach the objectives of developing new and successful anti-tuberculosis drugs in the near future. Research and development is also needed on innovative drug formulations and drug delivery systems aimed at increasing compliance and achieving a high local drug concentration while minimizing systemic side effects. In this respect, the growing field of inhalation therapy offers a very promising new prospect. This technology – presented as a simple, low-cost, disposable, dry-powder inhaler – can be applied to the delivery of anti-tuberculosis drugs.

On vaccine development

Vaccine development is a problematic issue for many reasons. It is not appealing for the industry, because it demands a huge investment, takes a long time and the risk of failure is high.

Moreover, major obstacles also lie in the initial vaccine design itself, such as the difficulty in inducing a potent and long-lasting cellular immune response in humans, due to our poor understanding of host-parasite interactions.

Future vaccines must prove able to protect against the most prevalent, transmissible and/or virulent lineages worldwide not against laboratory-domesticated strains.

A new promising approach is based on the fact that viral vectors, such as poxviruses, are powerful at boosting previously primed T-cell responses against intracellular pathogens. The vaccine candidate is now in clinical trials. Results from one of these trials showed that the recombinant viral vector vaccine is a strong booster of BCG-primed and naturally acquired antimycobacterial immunity. In fact, this is the first clinical trial showing successful results with a novel subunit TB vaccine.

A provocative finding was reported by researchers at the Institute Pasteur, where the BCG vaccine was first developed in 1924. They gathered a large body of evidence of a gradual loss in immunogenicity and protection ability. The findings suggest that the early BCG strain may even be superior to the later ones that are currently much more widely used BCG strain. It is speculated that, in general, vaccine manufacturers tended to prefer BCG strains that cause less serious local reactions over those provoking more intense inflammation at the site of injection. As a result, less protective strains for vaccine production might have unintentionally been selected through time.

New TB vaccine regimens are not expected to be introduced into national TB programs before 2030.

The tubercle bacillus is both an amazing creature and a formidable enemy that has proven hard to conquer. It is pushing science to its limits. Medical research itself has developed into a complex and engaging living creature whose evolution is driven by selective pressure. The greater the challenge the more eager the endeavor must be. We just can not afford to lose this battle.