Non-Bacteriologic Diagnosis of Tuberculosis Immune Response Indicators. Part II

Diagnóstico no bacteriológico de la tuberculosis. Indicadores de la respuesta inmune. Parte II

González, Claudio D.[1]; Fruhwald, Gladys E.[2]; Duré, Roberto M.[3]; Armitano, Rita I.[4]; Amiano, Nicolás O.[5]; García, Verónica E.[6]; Cerqueiro, María Cristina[7]

TB Diagnostic Study Group

Amiano, Nicolás O.[5]; Armitano, Rita I.[4]; Bisero, Elsa D.[8]; Cerqueiro, María Cristina[7]; Duré, Roberto M.[3]; Fruhwald, Gladys E.[2]; García, Verónica E.[5], González, Claudio D.[1]; González, Norma E.[9]; Lombardero, Lorena A.[7]; Luque, Graciela F.[7]; Melillo, Karina C.[7]; Símboli, Norberto F.[9]

1. DIAGNOSIS BASED ON CLINICAL CRITERIA

Claudio D. González

The system of symptoms and signs most widely used for the screening for suspected tuberculosis (TB) was provided by the WHO (World Health Organization). The presence of fever, night sweats, weight loss, and cough correlates with 77% of sensitivity and 68% of diagnostic specificity in HIV reactive patients.[1] Furthermore, in HIV non-reactive patients, a comparison has been made between the diagnostic capacity of the symptoms (cough, hemoptysis, fever, night sweats or weight loss), of the radiology, and the rapid diagnostic molecular tests, such as Xpert, LAMP and Truenat. Any of the said TB symptoms reached 71% sensitivity and 64% specificity; radiologic anomalies had 85% sensitivity and 98% specificity, and rapid tests in adults at risk reached 69% and 99%.[1]

2. DIAGNOSIS BASED ON CHEST IMAGING

The use of simple chest X-ray has been recommended a long time ago for the screening of the population with suspected TB. The sensitivity of the radiology in the diagnosis of pulmonary TB oscillates between 87% and 98%, and

[1] Pneumophthisiology Unit, Hospital General de Agudos José M. Ramos Mejía, Autonomous City of Buenos Aires, Argentina.

[2] Pulmonology Service of OSPERYH (Health Insurance for Rental and Horizontal Property Workers).

[3] Bronchoscopy Unit, Hospital de Infecciosas Francisco J. Muñiz, Autonomous City of Buenos Aires, Argentina.

[4] Laboratory for Mycobacteria. Hospital General de Agudos Parmenio Piñero, Autonomous City of Buenos Aires, Argentina.

[5] Researcher at CONICET (National Scientific and Technical Research Council). Laboratory of Immunity and Tuberculosis of the IQUIBICEN (Institute of Biological Chemistry, Faculty of Exact and Natural Sciences), University of Buenos Aires (UBA), Autonomous City of Buenos Aires. Argentina.

[6] Consulting Physician in the Department of Phthisiology. Hospital de Niños Dr. Ricardo Gutiérrez, Autonomous City of Buenos Aires, Argentina.

[7] Pediatric Service. Pediatric Pulmonology Department, Hospital Nacional Prof. Dr. Alejandro Posadas, El Palomar, Province of Buenos Aires, Argentina.

[8] Pneumophthisiology Unit, Hospital General de Niños Pedro de Elizalde, Autonomous City of Buenos Aires, Argentina.

[9] Mycobacteria Service, National Institute of Infectious Diseases - ANLIS Dr. Carlos G. Malbrán, City of Buenos Aires, Argentina.

the specificity is approximately 75%.[2] As additional information, it is observed that radiological findings can appear even before the four mentioned symptoms. The validity of simple chest radiology is explained by the fact that at least 90% of patients had evident radiological manifestations which are then confirmed in their bacteriology.[2, 3]

Regardless of the accesibility of the radiological resource, in certain groups it may be necessary to perform large-scale population screening. For that purpose, the Stop TB Partnership initiative recommends for the first time the use of three specialized software products of detection computerized assistance (CAD, computer aided detection) based on artificial intelligence that provide an automated and standardized interpretation of chest digital X-rays for radiologists or teleradiographs. Results are expressed as abnormality scores; the software may be used for detection or screening and is limited to simple X-rays for pulmonary TB in individuals of 15 years or older .[4, 5]

The section about TB diagnosis in children describes the usefulness and limitations of radiology in the diagnosis of TB.

3. DIAGNOSIS OF TB IN CLINICAL POINTS OF CARE (POC)

Gladys Esther Fruhwald

With the need to facilitate the access of patients to TB diagnostic methods, this system of decentralized care (POC) has been suggested.[6] It requires minimum staff training and simple diagnostic equipment with rapid results.

For the purpose of obtaining early detection of the disease, specially in vulnerable patients such as HIV carriers or children, the WHO proposes getting a new system that allows for a diagnosis without sputum samples or with the use of biomarkers. It also recommends a screening test that allows for the identification of those who need more tests, possibly sputum, blood or urine samples. In any case, the method replacing the sputum sample should have a sensitivity that can be comparable to the XPERT system; it has to be simple and, if possible, it shouldn't require any power or temperature control.[7]

Screening tools to identify who needs more tests can be used by taking into account the symptoms (cough, night sweats, weight loss, fever and hemop-

tysis) or the chest X-rays.[8] In the screening through symptoms, risk populations should be categorized by community (impoverished neighbourhoods, immigrants and people with direct contact with cases of TB), hospital departments and primary care centers; in the screening of people with history of HIV disease, undernourished individuals, diabetic patients or other immunosuppressed groups, place of residence (detention centers, shelters, immigration centers), and workplace (activities with high risk of having TB such as mining, health workers, etc.).[9]

Screening through portable chest X-ray is a simple, highly sensitive method, as shown by the experience in Kenya, where 92% sensitivity was obtained in patients with HIV and 100% in patients without that disease, with a specificity of 73%.[10] In this case, the X-ray is more sensitive than the screening through symptoms, specially if any of those symptoms is taken into account.

Finally, it is necessary to highlight the fact that in the diagnostic algorithms designed with the use of these simple resources, the X-ray is most useful when placed at the beginning of the algorithm.[11] The combination of screening through symptoms with imaging screening would allow compensating the lack of information of the latter, which can be missing or get lost, and seems to be more common precisely among the most vulnerable populations. The addition of a confirmation molecular test seems to be the ideal strategy for obtaining early TB diagnosis in POC units.[12] The use of a digital radiology system of automated reading could help reduce the bias in the interpretation of the technician in charge.[13]

4. DIAGNOSIS THROUGH INVASIVE METHODS

Roberto Miguel Duré

4.1 Role of bronchoscopy in the diagnosis of TB

Below is a description of four groups with risk of showing pulmonary TB, with indication of further evaluation through bronchoscopy.[14]

i. Patients with primary forms, HIV-negative, usually children.

ii. Patients with primary or post-primary forms, HIV-positive, generally with atypical radiological presentation.

iii. Patients with typical, post-primary forms, HIV-negative.

iv.   Patients with treatment failure and non-conclusive sputum samples, with suspicion of resistance to treatment drugs.

Patients from group 2 have a higher risk of disseminating their condition and also the possibility of other disease markers, that is why the use of this resource is more urgent in these patients. In adult patients from group 3, the risk of transmission will necessarily be low and the possibility of beginning an empiric treatment, according to the previously exposed criterion, should be evaluated. Finally, in children, gastric lavage should be performed as a first option before an endoscopy, and the decision to begin treatment shouldn't be delayed in this risk group.

This analysis would allow for the indication and rational use of the endoscopy in negative pulmonary forms, without disregarding the risk of bad evolution of each group.[15]

Indication of bronchoscopy according to clinical presentation

The clinical forms in which the endoscopy has proven effective are:
i.   Miliary TB, generally detected in group 2 and, to a lesser extent, in patients from group 3.
ii. Forms of intrathoracic tuberculous lymphadenopathy, commonly observed in groups 1 and 2.
iii. Other typical or atypical radiological forms, generally related to group 3.
iv. Asymptomatic patients with history of contact with TB focus and CT compatible with a tree-in-bud image. This is a new indication related to topographic image analysis. Although there isn't any evidence to conduct an invasive study, isolated cases being found will probably justify the use of that kind of study in the future.[15, 16]

For these forms of presentation, the available procedures are: the bronchial brush (BB), bronchial biopsy (BBy), transbronchial needle aspiration (TBNA), transbronchial biopsy (TBB) and bronchoalveolar lavage (BAL).[17-24]

Bronchial brushing (BB)

The use of the BAL has been extended more than the BB for the diagnosis of TB. However, the performance of the swab plus the culture studies is between 43% and 57%.[15, 16]

Bronchial biopsy (BBy)

The performance of the bronchial biopsy with some of the cited endoscopic images is 53%.[42] Although the prevalence of endobronchial TB is low (2.5% of the cases), we must mention that the most common forms, the caseous, the hyperemic-edematous and granular forms may evolve to the fibroestenotic form, or resolve completely within the first three months of treatment.[18, 19]

Transbronchial needle aspiration (TBNA)

It is recommended for mediastinal ganglionar forms, especially in right paratracheal, right and subcarinal bronchial and hiliar groups, in that order, because those are the most accessible for transbronchial needle aspiration.[19] At present, histology needles N° 19 such as the Wang or Schiepatti are suggested. In a study of 84 HIV-negative patients, Bilacerogu reaches 75% of the diagnosis with that method, adding the histological aspect with the biopsy culture (histological examination, 57% of efficacy *per se*).[21] With predominantly right nodes, the sensitivity is 83%, specificity 100%, NPV (negative predictive value) 38%, PPV (positive predictive value) 100% and precision of 85%[21]. The usefulness of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS - TBNA) isn't well-known yet as a diagnostic method of TB, but it could increase the profitability of needle aspirations.[22]

Transbronchial biopsy (TBB)

The miliary forms or segmental infiltrates have high specificity in the diagnosis of TB, and a sample shall always be sent for histological examination (5 samples) and culture. The performance of the TBB in TB is 73%, mainly based on the histological result.[21]

Bronchoalveolar lavage (BAL)

BAL is the most used endoscopic procedure for the diagnosis of pulmonary TB with negative sputum.

In comparative studies, the BAL contributed to the TB diagnosis in 30%, compared to 21% for gastric lavage and 16% for post-bronchoscopy sputum in a sample of 215 patients.[23]

The BAL had 89.7% sensitivity, 100% specificity, 100% positive predictive value, 94.6% negative predictive value and 96.3% test precision in suspected

cases of pulmonary TB with negative sputum/swab and culture.[24]

4.2. Diagnosis through measurement of adenosine deaminase enzyme

Rita Armitano

Pleural TB is the most common extrapulmonary manifestation of the infection caused by bacteria of the *M. tuberculosis* complex, and occurs with variable frequency according to each country in up to 30% of patients, regardless of coinfection by HIV.

The anatomopathological study and culture of the pleural biopsy are the diagnostic methods of choice. The histopathology shows a sensitivity of 56%-78% and a specificity of 95%, whereas the culture has a sensitivity that oscillates between 69% and 97% and a specificity of 96%.[25]

The adenosine deaminase (ADA) test in pleural fluid is a useful diagnostic examination, especially in patients who come from an environment with high prevalence of TB. There are numerous studies supporting its determination as a supplementary test for the diagnosis of tuberculous pleurisy.

The ADA enzyme participates in the catabolism of purine bases, the proliferation and differentiation of lymphoid cells and the maturation of macrophages. That enzyme is produced by monocytes and macrophages that catalyze the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. The level of ADA in pleural fluid reflects the presence of cells in the pleural cavity, mainly activated T-lymphocytes.[26]

At present, the reference method is the one described by Giusti, based on the detection of ammonia released in the enzymatic reaction and its subsequent quantification from a coloured compound.[26]

The determination of the parameters for the ADA test, like in any other diagnostic test, is in direct relation to the prevalence of the disease in question and other diseases that could influence the population being studied, to the study design and the methodology. Consequently, there are different discrimination values (cut-off points) for this test which according to the data published in the international literature vary from 30 U/L to 80 U/L. In accordance with the national recommendations based on a study conducted by the National Network of TB Bacteriology, where 152 patients with tuberculous pleural effussion were investigated through the manual colorimetric method of Giusti-Galanti, an ADA value of ≥ 60 U/L would have a sensitivity of 84% and a specificity of 94% for the diagnosis of tuberculous pleurisy.[27] In agreement with this work, the Mycobacteria Service INEI-ANLIS Carlos G. Malbrán, has shown that said cut-off level groups 80% of patients with pleural TB, therefore an inferior result wouldn't discard the diagnosis. One disadvantage of this method is the presence of false positives, for example in the case of non-tuberculous empyemas, the malignant proliferation of T-cells, systemic lupus erythematosus and rheumatoid arthritis pleurisy. In any case, the result will have to be analyzed according to the reference clinical situation, always considering the fact that diagnostic certainty requires microscopic examination and culture that confirm the presence of the *M. tuberculosis* complex.[27]

A study conducted in a reference laboratory of the Tuberculosis Care Network of CABA during 2016 evaluated the performance of an automated method for determining the presence of ADA in pleural fluid.[28] A total of 26 samples from patients with suspicion of tuberculous pleurisy were processed. The samples were divided in two aliquots. One of those aliquots was referred to the Mycobacteria Service INEI-ANLIS Carlos G. Malbrán for determination through the Galanti-Giusti manual colorimetric method, and the remaining aliquot was processed by the automated method DIAZIME with the COBAS 6000 - Module C50 analyzer, according to the manufacturer's instructions, at the Central Laboratory of the Hospital General de Agudos Parmenio Piñero.[28] The cut-off values were those of the Galanti and Giusti method: 60 UI/L 37°C, and DIAZIME method: 30 UI/L 37°C. In order to calculate the Kappa index, the mean values of each sample were classified in the following agreed categories: negative Galanti and Giusti method: ≤50 UI/L 37°C; borderline value: 50-70 UI/L 37°C; positive value: ≥70 UI/L 37°C. DIAZIME method: negative value: <29.4 UI/L 37°C; borderline value: 29.4-30.4 UI/L 37°C; positive value: >30.4 UI/L 37°C.

18 (69.2%) of the 26 processed samples were negative and 8 (30.8%) were positive, with both methods. No borderline results were obtained through any of the methods. The concordance strength in the classification per categories between the two used methods was excellent ($k = 1.000$).

These results suggest the usefulness of the automated method for the determination of ADA in pleural fluid, because apart from its high concordance with the reference method, no false positives or negatives were detected in this work.[28]

Another advantage of ADA determination is the fact that it is a simple method, easy to use and fast, with a mean result time of 2 hours, a value that can be reduced even further with the use of automated methods such as the one previously mentioned, with the possibility to increase the number of analyzed samples during a working day. Apart from the cases of false positives and negatives mentioned before, we must consider the alterations in the results caused by difficulties in the conservation and transport of the sample, the presence of hemolysis and exposure to high temperatures as other potential disadvantages. There is one limitation of the ADA research that we must emphasize: the method doesn't have an acceptable precision regarding the cerebrospinal fluid and other serous collections, due to the narrow range between normality and the cut-off points suggested for these extrapleural samples .[28]

4.3.   Diagnosis of infection through the interferon gamma assay (IGRA)

Nicolás Amiano and Verónica García

The latent infection caused by *M. tuberculosis* (LTBI) is a subclinical infection defined on the basis of the cellular immune response against mycobacteria antigens. The identification of the LTBI is important for the implementation of public health policies related to the control of the disease through the identification of individuals with high risk of developing active TB. Currently there isn't any assay that could be used as reference method (gold standard) for the identification of LTBI. The low bacterial load in tissue associated with LTBI prevents any diagnosis focused on the identification of the bacteria or its components. So, the diagnosis of LTBI consists in showing the cellular immune response of the individual against microbacterial antigens. In Argentina, the test to diagnose LTBI uses the purified protein derivative (PPD), but in the last years, developed countries implemented the interferon gamma release assays (IGRAs). These tests came from the search of antigens in exclusive regions of the *M. tuberculosis* genome (not present in *M. bovis*, BCG or any other mycobacteria species) as the main tools for developing new diagnostic methods. The basis of these assays lies in the fact that T cells from individuals previously sensitized with *M. tuberculosis* release interferon gamma (IFN-□) when being restimulated with pathogen-specific antigens; the most used ones are CFP-10 and ESAT-6.[29]

Since patients with active TB are infected with *M. tuberculosis*, they have been used as standard for the IGRA and PPD in order to determine the sensitivity of these assays; generally, the IGRAs are more sensitive than the PPDs. Therefore, in patients with active TB the skin test is usually positive, almost in 70% of the cases, whereas the IGRAs are positive in 80%-85% of the cases. Is this the real sensitivity of these diagnostic tests of latent infection? Maybe not, because the state of the immune system of individuals who progressed to active disease is different from that of subjects with latent infection, and this could affect the results of the assays that are based on the performance of cell-mediated immunity to detect previous exposure to *M. tuberculosis*.[30]

The IGRAs that are available in the market are the following:

I.   T-SPOT.TB (Oxford Immunotech, UK). With this method, the mononuclear cells of peripheral blood obtained by centrifugation are stimulated by CFP-10 and ESAT-6 for 16-24 h, and the ELISPOT technique analyzes the number of points (spots) indicating activated T cells that are producers of IFN-γ against the antigens.

II. QuantiFERON-TB Gold (QFT-QIAGEN, U.S.A, Germany) and QuantiFERON-TB Gold-Plus (QIAGEN, U.S.A, Germany). With this method, a sample of peripheral blood from the individual is stimulated with specific *M. tuberculosis* antigens for 16-24 h. Then, centrifugation is performed and plasma IFN-γ levels are determined through ELISA (enzyme-linked immunosorbent assay).

III. LIOFeron®TB/LTBI (LIONEX GmbH, Germany). The performance of this test is similar to that of QFT-QUIAGEN.

IV. VIDAS® TB-IGRA (BIOMÉRIEUX, France)

However, neither the PPD nor the available IGRAs allow the discrimination between active and latent TB. Also, these tests can't be used to predict if an individual with LTBI will be developing active TB or if the treatment for LTBI is effective in reducing the risk of developing active TB. An analysis carried out with 167 individuals (patients

with TB and persons cohabiting with patients) from hospitals in the city of Buenos Aires showed 78% concordance between QFT and PPD and 22% discordance (Kappa = 0.530 SE of kappa = 0.067), indicating a moderate strength of concordance.[31]

IGRA for the identification of infected contacts

The follow-up of the contacts of TB patients and the identification of subjects with LTBI after exposure to individuals with active TB are important elements of TB control. Several studies have provided different estimations of the rate of progression to active disease two years after the conversion of PPD/IGRA, but the general lifetime risk that is normally described accounts for 10%-15%.[32] Even though certain studies have suggested a higher risk of progression to active TB after a positive IGRA result, this difference wasn't significant in comparative meta-analyses. Therefore, the PPD or IGRA could be used to investigate contacts of active TB. Nevertheless, in populations whose contacts have a history of vaccination against BCG (bacille Calmette-Guerin), the highest specificity of the IGRA could allow a better orientation of preventive therapy. Still, it is important to highlight the fact that the specificity of the PPD is minimally affected by immunization with BCG if the vaccine is administered before one year of age.[33]For that reason, the IGRAs could be used in adults exposed to patients with active TB (for example, for the follow-up of the contacts) and the results would be more reliable in contacts vaccinated with BCG after one year of age.

IGRA to identify latent infection in immunocompromised patients

HIV infection increases the risk of LTBI progressing to a clinical disease in a significant way. Several studies confirm that the sensitivity of the IGRAs is reduced in subjects infected with HIV, with similar findings for PPD.[34] Low counts of CD4 T lymphocytes (<200 cells/μL) are associated with negative or indeterminate IGRA results. But some meta-analyses suggest that the T-SPOT.TB test has more sensitivity than the QFT in HIV-positive subjects for the diagnosis of active TB. On the contrary, other studies show that none of the existing IGRAs has proven to be more sensitive than PPD for the detection of LTBI in HIV-positive patients, and that IGRAs generally work in a way similar to PPD tests.[35]

Patients with immune-mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis, ulcerative colitis and Crohn's disease, inter alia, have an increased risk of developing active TB due to the immunosuppressive therapy they receive. Several studies have shown that IGRAs do not seem to be better than the PPD tests for the diagnosis of LTBI in patients with IMIDs.[36] However, more formal meta-analyses or longitudinal studies about the risk of active TB in these patients with negative and positive IGRAs should be conducted.

In our country, a diagnostic method of the IGRA type has been developed at the Laboratory of Immunity and Tuberculosis (IQUIBICEN, CONICET - UBA) (*Diagnos-TB*) that is similar to QuantiFERON but better, thanks to the addition of an extra tube that allows differentiating individuals with LTBI from healthy individuals, patients with TB and individuals recently exposed to *M. tuberculosis*, with 79% sensitivity and 83% specificity.[31] This method will be offered to the community in the future as High-Level Technological Services (STAN [Servicio Tecnológico de Alto Nivel, for its acronym in Spanish] - IQUIBICEN: www.iquibicen.fcen.uba.ar) at a cost clearly lower than imported IGRAs. At the same time, the paperwork for the ANMAT (National Administration of Drugs, Foods and Medical Devices) approval will be prepared.

5. THE FUTURE OF TB DIAGNOSIS. RESEARCH METHODS

María Cristina Cerqueiro

The translational medicine of this century has brought a major breakthrough in the diagnosis of TB, thanks to the movement of discoveries from basic research to medical practice. This is a slow process that usually poses a whole lot of challenges. In so far as new tests arise, it is not enough to identify variants of statistical significance; the clinical relevance must be supported in terms of validity and usefulness by profitability, noninvasiveness, efficacy and risk reduction. For that purpose, the WHO uses the criteria of the Target Product Profile (TPP).[7] For example, in order to identify disease progression, the method or element is required to reach minimum sensitivity precision $\geq$

75% and specificity ≥ 90%; for a new diagnostic test, 65% sensitivity and 98% specificity are expected; for the screening, a minimum sensitivity ≥ 95% and specificity ≥ 80%, with a cost ≤ USD 2 for a test not based on sputum that can be applied in the POC.

In order to improve the quick TB diagnosis, new tools are of crucial importance: noninvasive tests not based on sputum, and portable and affordable devices to apply those tests in a simple way, apart from the improvement in already existing tests. Translational research reinforces progress preventing and managing the whole TB spectrum; there is an urgent need for investment and support to strengthen the research capacity and its application in the healthcare field.

The TB diagnosis could be improved, specially in places with higher incidence of this disease, with easily accessible clinical samples, such as urine, stool, oral swabs, exhaled air or aerosols and the use of POC tests that do not require a source of energy, and are cheap and easy to use .[37] Also, such diagnostic tests must be widely applicable to every type of population, including children and immunocompromised individuals who frequently show negative sputum or tend to have atypical presentations, compromising the performance of the quickest NAATs (nucleic acid amplification tests).

THE "OMICS" PLATFORMS AND THEIR APPLICATION TECHNIQUES

The TB diagnosis requires the knowledge of the complex group of host-pathogen interactions, a process that has not yet been completely elucidated. Scientific breakthough related to such knowledge and the application of technologies has facilitated the development of tools and platforms of the whole biological system. These "omics" approaches are used in search of diagnostic tests for the tuberculous infection, its progression to disease, to monitor treatment efficacy and results, and to improve the understanding of the pathogenesis of the disease and its virulence in the application of vaccines and new treatments.[38]

Since 2015, breakthroughs in **genomics** have allowed the use of the whole genome sequencing (WGS) and the discovery of new biological mechanisms in TB. Unlike the serological techniques, this diagnostic tool can discriminate between TB reinfection and relapse , confirm the presence of current infection, and provide information for the epidemiological characterization and tracing of transmission.[39]

Epigenomics studies changes in the function of the genes without changing the sequence, for example, the non-specific effects of the Calmette-Guérin bacillus or the Mendelian susceptibility to mycobacteria.[40]

Proteomics researches the dynamics of protein products coded by the genome (proteome), ultimately facilitated with protein mass spectrometry (MS).[41]

The **transcriptomics** approaches research the patterns of genic expression to derive molecular signatures from the host and the physiology of the pathogen. They use RNA quantification methods, such as reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and RNA sequencing (RNA-Seq). They analyze ribosomal RNA (rRNA) to detect the viability of the bacillus, the gene regulation of micro RNA (miRNA) with the next-generation sequencing (NGS) and also the cellular signatures obtained from databases of gene ontology (GO) and flow cytometry.[42]

The infection of the immune cells of the host caused by *M. tuberculosis* causes several changes in metabolism; the metabolism of glucose and lipids is fundamental to defining the fate of the host cell function within the context of mycobacteria survival inside the granuloma.[39] **Metabolomics** focuses on understanding the interactions that occur in the disease environment. It allows the identification of metabolites with active and passive effects on phenotypes of interest, characterizes the metabolites of small molecules in the biological systems and their participation in various biological processes: cell differentiation and maturation, insulin signaling, T cell survival, energy transfer, immune responses of macrophages and cell-to-cell communication .[43] The **MS** and nuclear magnetic resonance (NMR) are the chosen techniques.

Fluxomics studies the dynamics of molecules, measures the metabolic phenotype of the biological system and provides an identification of carbon and nitrogen flow in the host.[38]

BIOMARKERS

The discovery of biomarkers can be based on different methodologies, such as the imaging techniques that show the fluctuation of a substance or the alteration in the structure and function (CAD,

biochemical techniques or omics techniques, to cite a few examples). A biomarker could be a cell or molecule that can be detected in a biological sample collected from the body that expresses *M. tuberculosis* exclusively or differentially or host molecules that express differentially in response to infection by *M. tuberculosis*.[39] One example of what is used currently in the field of TB would be the IFN-γ quantified by the IGRA, the LAM (lymphangioleiomyomatosis) antigen in urine or the Koch bacillus in the bacilloscopy.

In the last 20 years, only a few of the thousands of biomarkers reported in the bibliography have offered promising tools for clinical decision-making in TB. The omics approach is a high-performance method that allows obtaining biomarkers of multiple dimensions in only one step.

Bacterial biomarkers can derive from the analysis of their genes (urinary cell-free DNA), transcriptomic profiling or proteomics signatures. ESAT, CFP-10, Rv3615c, Rv3798c, MPT64, lipoproteins, mycolic acids or antigens in volatile compounds are detectable in sputum, plasma, expired air or urine.[39, 40]

Exosomes derived from *Mycobacterium tuberculosis* (Mtbexo) are a type of bioactive vesicle produced by the internal budding of endosomes, and are present in biological fluids. Preliminary evidence suggests that exosomes play a role in cell-to-cell communication and modulate immune and inflammatory responses of the host.[39, 44]

Host biomarkers study cellular immune signatures with high diagnostic sensitivity and specificity for active TB and distinguish the active from the latent infection, antigen expression in T cells, activation, memory, or proliferation markers with the participation of interleukins, various cytokines, the HLA-DR level or enzymatic molecules that intervene in signaling routes.[39, 40]

The genes and signatures or transcriptional records of RNA, of various materials, could distinguish the latent infection from active TB and predict disease progression.[42] For example, the combination of monocytes and macrophages and their relative expression of miRNA provide a more recent vision of the mechanism that generates the survival of the bacillus, the manipulation of the host's defense and the origin of latent infection and disease resistance.

The multi-omic integration, which represents multiple levels of biological organization, allows a more precise reconstruction of dynamic molecular networks that sustain healthy and sick states, using artificial intelligence applications with a variety of statistical and automatic learning approaches (machine learning).[42]

From the molecular diagnosis systems, lateral flow chromatographic assays, plasmid-based technology, and the volatile gas analysis (Aenose) to artificial intelligence processing, the search for future TB biomarkers shall be based on the principle of "patient-centered medicine" proposed by translational medicine.

DEVICES

In the last years, the development of research in POC diagnostic platforms has shown many advantages in its down-scaling process. The micro-nanodevices based on LOC platforms (Lab-on-a-chip) with microfluidic techniques also show high sensitivity, high-performance and accurate results, as well as low cost and portability in a compact format.[44] They are still in their first stages, like the **lateral flow chromatographic immunoassay (LFA)**, that uses porous membrane and other portable nanotechnologies (Oxford Nanopore Technologies-ONT).

Paper-based analytical devices (μPAD electrochemicals) represent the largest part of POC devices because paper is a low-cost, biocompatible substrate with high feasibility to integrate different function modules, thus favouring their use in the diagnosis of biological samples and the identification of biomarkers. Other devices include the development of **bioassays**, flow **cytometry**, Luminex bead-based **assays** and **multiple electro-chemioluminiscent immunoassays** (Meso Scale Discovery, MSD), which have achieved great success in the detection of multiple cytokines in serum and plasma samples.[44]

Aptamers, in turn, are short, single-stranded nucleic acids that fold into specific three-dimensional structures, and this allows them to bind to target molecules through different processes, for example, IFN 	 detetion with graphene-field effect transistors (GFETs) or with LFA.[45]

The clustered Regularly Interspaced Short Palindromic Repeats assay (CRISPR) that uses the trans-excision activity **CAS** (SHERLOCK and DETECTR) is a genomic edition method that functions as molecular scissors, cutting and modifying

bacterial DNA with a high degree of precision and specificity; its discovery received the Nobel prize in 2020 .

CRISPR-MTB detects *M. tuberculosis* directly in clinical samples, including sputum, BAL, CSF, pleural fluid, ascites and pus with improved sensitivity (that is to say, with an almost unique copy); it requires lower sample volume and provides faster results. CRISPR/Cas9 and CRISPR/Cas12a detect DNA or RNA and are viewed with blue luminescence signal. Also, cytokines have been detected which are viewed with LFA.[46]

Large-scale use of new diagnostic tests in clinical applications and public health is yet limited by a series of factors: lack of standardized solutions, technical requirements of the laboratories, high costs associated with the use and maintenance of these molecular technologies, Internet infrastructure and cloud computing.[47]

We hope that addressing these barriers improves patients' results, but we still need a real POC test to meet the remaining challenges, such as sample preparation and human resources demands.

Conflicts of interest. The authors of this work declare that there is no conflict of interest.

REFERENCES

1. WHO operational handbook on tuberculosis. Module 2: screening - systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021. https://www.who.int/publications/i/item/9789240022614

2. World Health Organization. Chest radiography in tuberculosis detection: summary of current WHO recommendations and guidance on programmatic approaches. Geneva: World Health Organization; 2016. WHO/HTM/TB/2016. https://www.who.int/publications/i/item/9789241511506

3. Frascella B, Richards AS, Sossen B, et al. Subclinical tuberculosis disease - a review and analysis of prevalence surveys to inform definitions, burden, associations and screening methodology. Clin Infect Dis. 2021;73:e830-e841. https://doi.org/10.1093/cid/ciaa1402.

4. Stop TB Partnership and FIND resource center on computer-aided detection products for the diagnosis of tuberculosis. AI4HLTH website. En: https://www.ai4hlth.org/

5. Qin ZZ, Naheyan T, Ruhwald M et al. A new resource on artificial intelligence powered computer automated detection software products for tuberculosis programmes and implementers. Tuberculosis 2021;127:102049. https://doi.org/10.1016/j.tube.2020.102049.

6. Schito M, Peter TF, Cavanaugh S et al. Opportunities and challenges for cost-efficient implementation of new Point-of-Care diagnostics for HIV y Tuberculosis. J Infect Dis. 2012;205(Suppl. 2):-S169-S180. https://doi.org/10.1093/infdis/jis044

7. World Health Organization. High-priority target produt profiles for new tuberculosis diagnostics: report of a consensus meeting. Genova, Switzerland: WHO/HTM/TB/2014.18. World Health Organization https://apps.who.int/iris/handle/10665/135617

8. WHO consolidated guidelines on tuberculosis. Module 2: screening – systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021. https://www.who.int/publications/i/item/9789240022676 74

9. Word Health Organization. Systematic screening for active tuberculosis: principles and recommendations. WHO/HTM/TB/2013.04. 31. Word Health Organization https://apps.who.int/iris/handle/10665/84971

10. van't Hoog AH, Meme HK, Laserson KF et al. Screening Strategies for Tuberculosis Prevalence Surveys: The Value of Chest Radiography and Symptoms. PLoS ONE 2012; 7(7): e38691. https://doi.org/10.1371/journal.pone.0038691

11. Theron G, Pooran A, Peter J et al. Do adjunct tuberculosis test, when combined Xpert MTB/RIF, improve accuracy and the cost of diagnosis in the resource-poor setting? Eur Respir J. 2012; 40:161-8. https://doi.org/10.1183/09031936.00145511

12. Philipsen RHHM, Sánchez Cl, Maduskar P et al. Automated chest-radiography as a triage for Xpert testing in a resource-constrained setting: a prospective study of diagnostic accuracy and cost. Sci Rep. 2015; 5:12215. https://doi.org/10.1038/srep12215. 35.

13. Hogeweg L, Sánchez CI, Maduskar P et al. Automatic detection of tuberculosis in chest radiographs using a combination of textural, focal, and shape abnormality analysis. IEEE Trans Med Imaging. 2015;34:2429-42. https://doi.org/10.1109/TMI.2015.2405761.

14. Long R. Smear negative pulmonary tuberculosis in industrialized countries. Chest 2001;120:330-4. https://doi.org/10.1378/chest.120.2.330

15. Willcox PA, Potgieter PD, Bateman ED, Benatar SD. Rapid diagnosis of sputum negative miliary Tuberculosis using flexible fibreoptic bronchoscope. Thorax 1986;41:681-4. https://doi.org/ 10.1136/thx.41.9.681.

16. Willcox PA, Benatar SR, Porgierter PD. Use of flexible fiberoptic bronchoscope in the diagnosis of sputum-negative tuberculosis. Thorax 1982;37:598-601. https://doi.org/10.1136/thx.37.8.598

17. Kim YW, Kwon BS, Lim SY et al. Diagnostic value of bronchoalveolar lavage and bronchial washing in sputum-scarce or smear-negative cases with suspected pulmonary tuberculosis: a randomized study. Clin Microbiol Infect. 2020;26:911-6. https://doi.org/10.1016/j.cmi.2019.11.013. 75.

18. Kumar R, Gupta N. Role of bronchoscopy in evaluation of cases with sputum smear negative pulmonary tuberculosis, interstitial lung disease and lung malignancy: a retrospective study of 712 cases. Indian J Tuberc. 2015;62:36-42. https://doi.org/10.1016/j.ijtb.2015.02.006.

19. Baran R, Tor M, Tahaoglu K, Ozvaran K, Kir A, Kizkin O, Turker H. Intrathoracic tuberculosis lymphadenopathy: clinical and bronchoscopic features in 17 adults without parenchymal lesions. Thorax 1996;51:87-9. https://doi.org/10.1136/thx.51.1.87

20. Chung HS; Lee JH. Bronchoscopic assessment of the evolution of endobronchial tuberculosis. Chest 2000;117:385-2. https://doi.org/10.1378/chest.117.2.385

21. Bilaçeroğlu S, Günel O, Eriş N, Cağirici U, Mehta AC. Transbronchial needle aspiration in diagnosing intrathoracic tuberculous lymphadenitis. Chest 2004; 126:259-67. https://doi.org/10.1378/chest.126.1.259

22. Lin SM, Chung FT, Huang CD et al. Diagnostic value of endobronchial ultrasonography for pulmonary tuberculosis. J Thorac Cardiovasc Surg 2009;138:79-84. https://doi.org/10.1016/j.jtcvs.2009.04.004.

23. Dickson SJ, Brent A, Davidson RN, Wall R. Comparison of bronchoscopy and gastric washings in the investigation of smear-negative pulmonary tuberculosis. Clin Infect Dis 2003;37:1649-53. https://doi.org/10.1086/379716

24. Ahmad M, Ibrahim WH, Sarafandi SA et al. Diagnostic value of bronchoalveolar lavage in the subset of patients with negative sputum/smear and mycobacterial culture and a suspicion of pulmonary tuberculosis. Int J Infect Dis. 2019;82:96-101. https://doi.org/10.1016/j.ijid.2019.03.021.

25. Shaw JA, Diacon AH, Koegelenberg CFN. Tuberculous pleural effusion. Invited Review Respirology. 2019;24(10):962-71. https://doi.org/10.1111/resp.1367 10:962-971.

26. Giusti G. "ADA in Bergmeyer H.V" ed, Methods of enzimatic analysis, New York Academic Press Inc, 1974; pp: 1092-1099.6. 76

27. Zerbini E, Imaz MS, R. Franco R et al. Utilidad de la determinación de la actividad de Adenosín Deaminasa en el diagnóstico de la tuberculosis extrapulmonar. IX Congreso Argentino de Microbiología, 2001

28. Armitano RI, Cordoma NS, Pantazis S, et al. Pleuresía tuberculosa. Evaluación del rendimiento de un método automatizado para la determinación de Adenosina Deaminasa en líquido pleural. VIII Congreso de la Sociedad Argentina de Bacteriología, Micología y Parasitología Clínicas SADEBAC - II Jornada de Micología Clínica - I Jornada de Parasitología Clínica, noviembre de 2018.

29. Carranza C, Pedraza-Sanchez S, de Oyarzabal-Mendez E, Torres, M. Diagnosis for Latent Tuberculosis Infection: New Alternatives. Front Immunol. 2020;11:2006. https://doi.org/10.3389/fimmu.2020.02006

30. Schluger, NW. Advances in the Diagnosis of Latent Tuberculosis Infection. Semin. Respir Crit Care Med 2013;34:60-6. https://doi.org/10.1055/s-0032-1333545

31. Amiano NO, Morelli MP, Pellegrini JM, et al. IFN-γ and IgG Responses to Mycobacterium Tuberculosis Latency Antigen Rv2626c Differentiate Remote from Recent Tuberculosis Infection. Sci Rep 2020;10:7472. https://doi.org/10.1038/s41598-020- 64428-z

32. Herrera V, Perry S, Parsonnet J, Banaei N. Clinical Application and Limitations of Interferon- Release Assays for the Diagnosis of Latent Tuberculosis Infection. Clin Infect 2011;52:1031-7. https://doi.org/10.1093/cid/cir068

33. Farhat, M.; Greenaway, C.; Pai, M.; Menzies, D. False-Positive Tuberculin Skin Tests: What Is the Absolute Effect of BCG and Non-Tuberculous Mycobacteria? Int J Tuberc Lung Dis. 2006;10:1192-204.

34. Cattamanchi, A.; Smith, R.; Steingart, K. R. et al. Interferon-Gamma Release Assays for the Diagnosis of Latent Tuberculosis Infection in HIV-Infected Individuals: A Systematic Review and Meta-Analysis. J. Acquir. Immune Defic. Syndr. 2011;56:230-8. https://doi.org/10.1097/QAI.0b013e31820b07ab 77

35. Pai M; Denkinger C M; Kik SV et al. Gamma Interferon Release Assays for Detection of Mycobacterium Tuberculosis Infection. Clin Microbiol Rev 2014;27:3-20. https://doi.org/10.1128/CMR.00034-13.

36. Smith R, Cattamanchi A, Steingart KR, et al. Interferon-γ Release Assays for Diagnosis of Latent Tuberculosis Infection: Evidence in Immune-Mediated Inflammatory Disorders. Curr Opin Rheumatol 2011; 23:377-84. https://doi.org/10.1097/BOR.0b013e3283474d62

37. Nathavitharana RR, Garcia-Basteiro AL, Ruhwald M, Cobelens F, Theron G. Reimagining the status quo: How close are we to rapid sputum-free tuberculosis diagnostics for all? EBioMedicine. 2022;78:103939. https://doi.org/10.1016/j.ebiom.2022.103939.

38. Borah K, Xu Y, McFadden J. Dissecting Host-Pathogen Interactions in TB Using Systems Based Omic Approaches. Front Immunol 2021;12:762315. https://doi.org/10.3389/fimmu.2021.762315

39. Kanabalan RD, Lee LJ, Lee TY et al. Human tuberculosis and Mycobacterium tuberculosis complex: A review on genetic diversity, pathogenesis and omics approaches in host biomarkers discovery. Microbiological Research 2021;246:126674. https://doi.org/10.1016/j.micres.2020.126674.

40. Kontsevaya I, Lange C, Comella-del-Barrio P, et al. Perspectives for systems biology in the management of tuberculosis. Eur Respir Rev 2021;30:200377 https://doi.org/10.1183/16000617.0377-2020]

41. Guo J, Zhang X, Chen X and Cai Y. Proteomics in Biomarker Discovery for Tuberculosis: Current Status and Future Perspectives. Front Microbiol. 2022;13:845229. https://doi.org/10.3389/fmicb.2022.845229

42. Kaforou M, Broderick C, Vito O, Levin M, Scriba TJ, Seddon JA. Transcriptomics for child and adolescent tuberculosis. Immunol Rev 2022;309:97-122. https://doi.org/10.1111/imr.13116.

43. Gong W and Wu X. Differential Diagnosis of Latent Tuberculosis Infection and Active Tuberculosis: A Key to a Successful Tuberculosis Control Strategy. Front Microbiol 2021;12:745592. https://doi.org/10.3389/fmicb.2021.745592

44. Hong JM, Lee H, Menob NV, Lim CT, Lee LP, Ong CW. Point-of-care diagnostic tests for tuberculosis disease. Sci Transl Med 2022;14(639):eabj4124. https://doi.org/10.1126/scitranslmed.abj4124.

45. Roy S, Arshad F, Eissa S et al. Recent developments towards portable point-of care diagnostic devices for pathogen detec-tion. Sens Diagn. 2022;1:87-105. https://doi.org/10.1039/d1sd00017a

46. Rahman R, Majumder TR, Apu AI, Paul AK, Afrose A, Dash BK. CRISPR-Based Programmable Nucleic Acid-Binding Protein Technology Can Specifically Detect Fatal Tropical Disease-Causing Pathogens. Journal of Tropical Medicine 2022; 5390685:1-12. https://doi.org/10.1155/2022/5390685

47. Abdulgader SM, Okunola AO, Ndlangalavu G et al. Diag-nosing Tuberculosis: What Do New Technologies Allow Us to (Not) Do? Respiration 2022;101:797-813. https://doi.org/10.1159/000525142