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Comparative study of the performance of GeneXpert *MTB/RIF* in bronchoalveolar lavage compared to bronchial lavage in patients with clinical suspicion of tuberculosis

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Abstract

Tuberculosis (TB) is one of the ten leading causes of death worldwide, and the main cause from a single infectious agent. Early detection of the *Mycobacterium tuberculosis* complex (MTC) and of mutations conferring resistance to the main drugs used in antituberculous treatment contributes to reducing the transmission of the infection, and consequently the spread of resistant TB. The GeneXpert *MTB/RIF* test identifies the MTC and simultaneously detects mutations most frequently associated with rifampicin resistance, through real-time PCR testing.

The purpose of this study was to compare the performance of the GeneXpert *MTB/RIF* method in bronchoalveolar lavage (BAL) with bronchial lavage (LB) in immunocompetent patients with clinical suspicion of pulmonary TB without any previous microbiological documentation.

Materials and Methods: We prospectively enrolled patients with radiologic pulmonary infiltrates compatible with active or residual TB without previous treatment, with negative direct bacilloscopy or nonproductive cough, for the assessment of active disease. We identified the most affected segment through computed axial tomography and bronchoscopy with BAL in said segment, followed by BL of the affected lobe. A BAL recovery > 40% was considered significant. The samples obtained were processed for bacilloscopy, culture and GeneXpert *MTB/RIF*. We analyzed sensitivity (S), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV), taking the solid culture medium as reference for the diagnosis of MTC.

Results: We included 20 patients; 3 were excluded because they didn't have a representative BAL sample. 17 patients were evaluated (11 women, 65%), age 37.2 ± 16.3. The MTC was identified through conventional methods in 10 patients: 10 with positive culture in BL and 9 in BAL.

In comparison with the conventional methods, 6 out of 17 samples obtained through BAL had a positive result for GeneXpert *MTB/RIF*: S = 60.0% (CI 31%-83%), SP = 100% (CI 65%-100%), PPV = 100% (CI 61%-100%) and NPV = 64% (CI 35.4%-84.8%). With BL, 9 out of 17 had a positive result for Xpert *MTB/RIF*: S = 90.0% (CI 60%-98%), SP = 100% (CI 65%-100%), PPV = 100% (CI 70%-100%) and NPV = 88% (CI 53%-98%). All the cases identified with GeneXpert MTB/RIF were true positives in relation to conventional cultures.

Conclusion: Considering the solid culture as reference method, the BL was more sensitive than the BAL for the diagnosis of tuberculous infection through the GeneXpert *MTB/RIF* method in patients with suspected TB without previous microbiological documentation.

Key words: Tuberculosis; Bronchoalveolar lavage; Comparative study

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Introduction

Tuberculosis (TB), a preventable and curable condition, is one of the most widespread infectious diseases around the world and constitutes an important threat to public health. There are approximately 2 billion people around the world infected with the *Mycobacterium tuberculosis* complex (MTC). Every year almost 9 million people develop the active disease and 2 million people die from it[1].

The microbiological diagnosis of TB is complicated. The bacilloscopy is a fast, simple, low-cost technique but has low sensitivity[2, 3]. Although the culture test is still the reference method or gold standard for the diagnosis of all types of TB because it allows for gender and species identification, it requires higher level laboratories with more demanding infrastructure and equipment conditions, and the results are obtained after 15 days and even 8 weeks of incubation of the clinical samples.

Early detection of the MTC and of mutations conferring resistance to the main drugs used in antituberculous treatment has a great impact on the management, prognosis and evolution of the disease apart from contributing to reduce the transmission of the infection, and consequently the spread of resistant TB[1-4].

In this regard, during the last years different molecular biology techniques have emerged noticeably accelerating the TB diagnosis in comparison with the use of bacilloscopy and culture.

The GeneXpert *MTB*/*RIF* test is based on the detection of MTC-specific nucleic acids through real-time PCR testing. The GeneXpert *MTB*/*RIF* simultaneously identifies the MTC and detects the *rpo*B gene mutations more frequently associated with resistance to rifampicin, the most important drug against this disease. Itis a completely automated process that allows the diagnosis within a period of two hours[2]. The WHO (World Health Organization) recommends this test for the initial diagnosis of TB[1-4].

In patients with clinical suspicion of TB and nonproductive cough or negative bacilloscopy, respiratory samples can be obtained for infectious analysis through bronchoscopy, providing an early diagnostic confirmation[5-9].

The flexible bronchoscopy is a diagnostic and therapeutic procedure essential for respiratory medicine[6,7]. Bronchoalveolar lavage (BAL) is the reference bronchoscopic sample for the study of infectious diseases that affect the pulmonary parenchyma[8, 9], and bronchial lavage (BL) is a complementary sample due to the possibility to be contaminated with secretions from the upper respiratory tract.

When the BAL is not available or the samples obtained are not significant for the analysis, the question arises as to consider the BL as the representative sample for the etiologic study in the situations previously mentioned.

The purpose of this study was to compare the performance of the GeneXpert *MTB*/*RIF* method in BAL with BL in immunocompetent patients with clinical suspicion of pulmonary TB, without previous microbiological documentation, in order to optimize the use of diagnostic resources.

Materials and Methods

This prospective, cross-sectional study was conducted at the Department of Respiratory Medicine and the Central Laboratory, Bacteriology Section of the *Hospital General de Agudos Parmenio Piñero*, within the period from June 2018 to December 2019.

All the bronchoscopy procedures were performed by the staff of the Respiratory Medicine Division. We used a videobronchoscope of the 70K series with a diameter of 6.2 mm (model EB - 1970K, PENTAX). All visible bronchi and pulmonary segments were examined through topical anesthesia; and samples were collected from the pulmonary segment or subsegment which showed abnormal lesions suggestive of active TB in the HRCT (High Resolution Computed Tomography).

For the BAL, 100 to 120 ml of saline solution 0.9% were instilled in five or six aliquots, the first being separated and attached to the BL. The BL was done after the BAL. 20-40 ml of saline solution 0.9% were instilled in the other pulmonary segments involved, and aspirated until 20 to 30 ml of liquid were collected in the suction trap: then the first aliquot obtained with the BAL was added.

We excluded patients in whom the BAL couldn't be performed or those who didn't have significant samples. A BAL recovery > 40% was considered significant. There weren't any complications during the procedure.

Samples obtained were divided and referred for bacteriological and cytological studies. At the Bacteriology Service each sample was divided in two: one part was used for identifying the MTC and detecting sensitivity to rifampicin by GeneXpert MTB/RIF, whereas the remaining aliquot was used for the bacilloscopy and culture in a liquid medium (MGIT 960, Becton Dickinson-BD) and in a solid medium (Löwenstein Jensen and Stone Brink).

We determined sensitivity (S), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV) taking the culture in solid medium, Löwenstein-Jensen and Stone Brink as method of reference.

Results

3 of the 20 patients included in the study (15%) were excluded because they obtained an insufficient volume with the BAL (Figure 1).

17 patients were evaluated (11 women, 65%), with a mean age of 37.2 ±16.3 years. Distribution per nationality was: 10 (59%) Argentinians, 6 (35%) Bolivians and 1 (6%) Peruvian. Most patients didn't show comorbidities (n = 15, 88%) and didn't have a history of TB (n = 16, 95%). The most frequently found lesions were infiltrates or consolidations in the upper lobes (n = 17, 100%).

The combination of the results of the BAL and BL samples allowed us to identify 10 patients with MTC through conventional methods (one with positive bacilloscopy in BL, 10 with positive cultures in BL and 9 in BAL); the remaining cases were clinically followed-up.

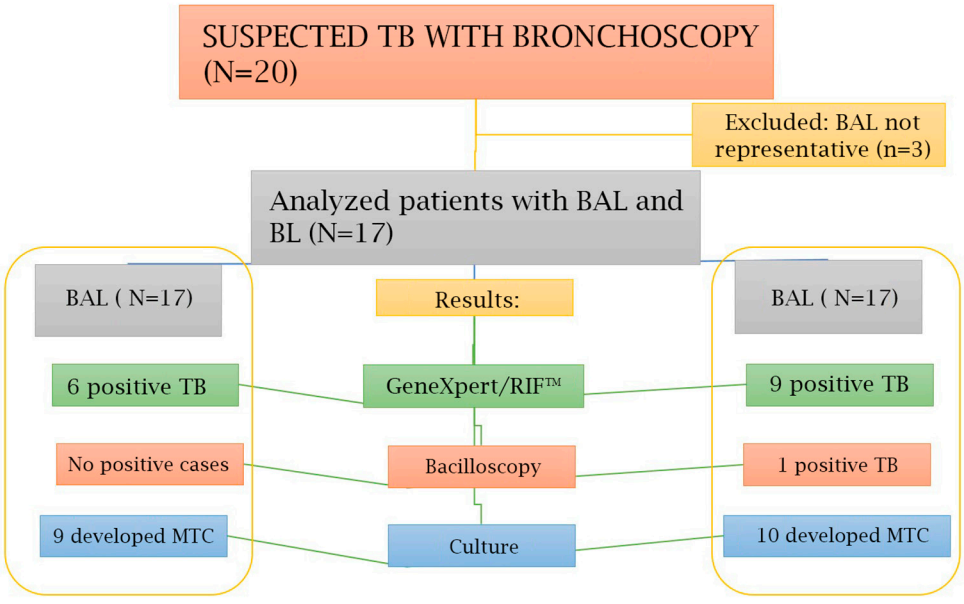

Figure 1. Flow of patients included in the analisys

6 out of the 17 samples obtained through BAL were positive with GeneXpert *MTB*/*RIF*: S = 60.0%, SP = 100%, PPV= 100% and NPV = 64%.

With BL, 9 out of 17 had a positive result for GeneXpert *MTB*/*RIF*: S = 90.0%, SP = 100%, PPV = 100% and NPV = 88%. All the cases identified with GeneXpert *MTB*/*RIF* were true positives in relation to conventional cultures. The results obtained are shown schematically and with confidence intervals in Table 1.

In this study we didn't assess the importance of the GeneXpert *MTB*/*RIF* test in detecting resistance to rifampicin in FBC (fibrobronchoscopy) samples, though they weren't detected in patients under evaluation.

TABLE 1. Comparison of the Xpert MTB/RIF performance

Bronchoscopy samples	Reference Positive culture 10/17	S %	E %	LR Positive	LR Negative	PPV % (CI)	NPV % (CI)
BAL	Positive PCR 6/17	60	100	0.6	0.4	100 (61-100)	64 (35-85)
LB	Positive PCR 9/17	90	100	0,9	0,1	100 (70-100)	88 (53-98)

BAL = bronchoalveolar lavage; BL = bronchial lavage, S = sensitivity, SP = specificity, LR = likelihood ratios

Discussion

The bronchoscopic diagnosis of pulmonary tuberculosis is traditionally based on bacilloscopy and MTC culture. The genomic analysis through nucleic acid amplification techniques such as the GeneXpert *MTB*/*RIF* allows for a rapid, high-sensitivity diagnosis.

Given the fact that WHO recommendations on GeneXpert *MTB*/*RIF* only refer to sputum samples, more research is necessary on the use of this PCR in bronchoscopic samples.

Le Palud et al, 2014 conducted a retrospective, observational study about the diagnostic precision of the GeneXpert *MTB*/*RIF* test in fibrobronchoscopic samples, where the type of sample was left to the discretion of the specialist according to the patient's tolerance to treatment[10].

In our work, the diagnostic performance of the BL turned out to be superior to that of the BAL for the identification of active pulmonary TB. With regard to other studies that analyze the BAL[11, 12] or the BL[13, 14] performance individually, we compared the performance of GeneXpert *MTB*/*RIF* in BAL and BL together in the same procedure in patients with clinical suspicion of TB and we considered the culture as reference method. We observed that the BAL was less sensitive than the BL for the diagnosis of TB (60% versus 90%, respectively), with the same specificity (100%).

Bronchial lavage is referred to in research done in Japan (Kohno, Kurashima and Takano), India (Arshad, Gupta[15]) and South Korea (Yoo, Song) as the reference sample for the study of TB, using BAL for other respiratory diseases. Those are centers with high incidence of TB.

One limitation to the work is the small sample of individuals included. Another observation regarding the procedure used for sample collection (BL after BAL, including the same segment already assessed with the latter) is that we could infer a better diagnostic performance of the BL when we include the residual material from the BAL.

Conclusion

Considering the solid culture as reference method, the bronchial lavage was more sensitive than bronchoalveolar lavage for the diagnosis of tuberculous infection through the GeneXpert *MTB*/*RIF* method in patients with suspected TB without previous microbiological documentation.

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