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Brief report

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Retrospective diagnosis of lymphatic tuberculosis in frozen samples using two genetic amplification methods, Xpert® MTB/RIF ULTRA and Abbott RealTime MTB Assay

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ABSTRACT

Objectives. The main objective of the present study is to assess the sensitivity and specificity of a retrospective diagnostic of lymphatic tuberculosis (LTB), testing frozen samples using gene amplification PCR methods. The secondary objective was to compare the results of two different commercial tuberculosis gene amplification methods for this purpose.

Material and methods. We retrospectively studied 38 frozen samples, previously processed for mycobacterial culture between January 2014 and August 2019. The results of the previous cultures were: 21 samples positive for *Mycobacterium tuberculosis* complex (MTB) (5 being smear positive), 7 samples culture positive for *Mycobacterium avium-intracellulare* complex and 10 samples which were mycobacterial culture negative and discarded for LTB diagnosis, used as controls. The samples were processed using two gene amplification methods: Xpert® MTB/RIF Ultra (Cepheid) and Abbott RealTime MTB Assay (Abbott).

Results. Compared to initial culture results the sensitivity and specificity of Xpert® MTB/RIF Ultra were 57.1% and 100% and 52.3 % and 92.5%, respectively for the Abbott RealTime MTB assay. The differences were not statistically significant. In addition, there were no differences according to the period of freezing.

Conclusions. Gene amplification of frozen samples confirmed the diagnosis of lymphatic TB in almost 60% of cases, allowing retrospective diagnosis in initially non suspected cases. Both gene amplification techniques tested were equally useful.

Keywords: Frozen lymph nodes samples, retrospective TB diagnosis, Xpert® ULTRA, Abbott RealTime MTB, lymph nodes, extrapulmonary tuberculosis.

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Diagnóstico retrospectivo de tuberculosis linfática en muestras congeladas utilizando dos métodos de amplificación genética, Xpert MTB/RIF ULTRA y Abbott RealTime MTB Assay

RESUMEN

Objetivos. El principal objetivo del presente estudio es establecer la sensibilidad y especificidad del diagnóstico retrospectivo de tuberculosis linfática (LTB), analizando muestras congeladas y utilizando métodos de amplificación genética basados en PCR. El objetivo secundario es comparar los resultados de dos métodos comercializados de amplificación genética de tuberculosis.

Material y métodos. Se estudiaron retrospectivamente 38 muestras congeladas, que previamente habían sido procesadas para realizar cultivo de micobacterias, entre enero de 2014 y agosto de 2019. Los resultados en ese momento fueron: 21 muestras positivas para *Mycobacterium tuberculosis* complex (MTB) (de las que 5 tenían la tinción positiva), 7 muestras con cultivo positivo para *Mycobacterium avium-intracellulare* complex y 10 muestras con cultivo de micobacterias negativo, en las que se había descartado el diagnóstico de LTB, que se usaron como controles. Las muestras se procesaron utilizando dos métodos de amplificación genética: Xpert® MTB/RIF Ultra (Cepheid) y Abbott RealTime MTB Assay (Abbott).

Resultados. Comparando con los resultados de los cultivos iniciales, la sensibilidad y especificidad de Xpert® MTB/RIF Ultra fueron de 57,1% y 100%, y de 52,3% y 92,5%, para Abbott RealTime MTB assay. Las diferencias no fueron estadísticamente significativas. Tampoco se observaron diferencias en relación al periodo transcurrido desde la congelación.

Conclusiones. La amplificación genética de muestras congeladas confirma el diagnóstico de tuberculosis linfática en casi el 60% de los casos, permitiendo el diagnóstico retrospec-

tivo de casos no sospechados. Las dos técnicas de amplificación analizadas demostraron la misma utilidad.

Palabras clave: muestras congeladas de gánglios linfáticos, diagnóstico retrospectivo de TB, Xpert ULTRA, Abbott RealTime MTB, nódulos linfáticos, tuberculosis extrapulmonar.

INTRODUCTION

According to the last report of the World Health Organization (WHO), ten million cases of tuberculosis (TB) were diagnosed during 2019. Moreover, it is estimated that 1.4 million people died of TB during this period [1]. Pulmonary tuberculosis is the most frequent form of clinical presentation of tuberculosis [1]. Extrapulmonary TB accounts for 16% of TB cases worldwide, with unequal distribution according to world region and HIV status of the patients. In the European WHO Region, extrapulmonary TB is found in 16% of the cases [1]. Extrapulmonary TB can affect any organ or tissue, although it is most frequently found in the lymph nodes and pleura and as disseminated forms in immunosuppressed patients [2]. Around 30 to 50% of extrapulmonary TB cases can affect lymph nodes, the most common localizations being lymph nodes in the cervical, submandibular or subclavicular neck area. Intrathoracic and intraabdominal nodes are less frequently affected, and sometimes not suspected as TB [2]. To diagnose lymphatic TB, especially localized in the neck, needle aspiration is performed and the sample undergoes cytological and microbiological study. Excision biopsy is performed when the needle aspiration results are negative.

The diagnosis of lymphatic TB is based on clinical symptoms and signs related to the localization and histological findings such as granulomatous reaction and/or caseous necrosis, and microbiological results. Microbiological tests comprise smear staining, mycobacterial culture and nucleic acid amplification tests (NAAT), when suspicion is high or moderately high. According to different studies, the diagnosis is confirmed in over 53-85% of the cases [2-4].

In recent years several commercial NAAT have been developed for the diagnosis of TB. Among the most used are two versions of the Xpert® assays (Cepheid, Sunnyvale, USA), the Xpert® MTB/RIF Assay and the Xpert® MTB/RIF Ultra. Both are integrated molecular tests able to detect *Mycobacterium tuberculosis* complex (MTB) and rifampicin resistance with minimal hands-on manipulation [4-7]. The ultra-version has greater sensitivity due to the incorporation of two new targets and improved polymerase chain reaction (PCR) parameters [7]. Another PCR-based test developed in the last years is the Abbott RealTime MTB Assay (Abbott, Chicago USA) [8].

The aim of the present study was to study the sensitivity and specificity of a retrospective diagnosis of lymph node TB analysing frozen samples using two NAAT in comparison to the initial mycobacterial culture.

METHODS

Sample collection. The mycobacterial laboratory of the hospital stores aliquots of remaining decontaminated samples processed for mycobacterial culture. Samples are maintained at -80ºC and thawed if required for PCR or repeating the culture. In the present study, a total of 38 aliquots of frozen decontaminated lymph node samples, processed for mycobacterial culture between January 2014 and August 2019 were selected and thawed. Twenty-one samples were obtained from tuberculous patients culture-positive for MTB, and 7 samples were obtained from patients suspected of mycobacterial disease who were culture positive for *Mycobacterium avium-intracellulare* complex (MAC). Ten mycobacterial culture-negative samples obtained from patients with a final diagnosis other than mycobacterial infection were included as controls.

The Xpert® MTB/RIF ULTRA and Abbott RealTime MTB assays were performed in the 38 samples included in the study. Before the amplification tests, a tissue digestion step was performed: 2 mL of the sample were centrifuged for 10 min, and the sediment recovered was then resuspended into 800 µL of digestion buffer with 40 µL of proteinase K (Qiagen, Hilden, Germany), followed by a heating at 56 ºC overnight. The digestion volume was adjusted to 1.1 ml and distributed as follows: 0.6 ml of the digested sample were tested for Xpert® MTB/RIF ULTRA using the GeneXpert instrument (Cepheid Sunnyvale, USA), and 0.5 ml were tested with the Abbott RealTime MTB. The results of both methods were interpreted according to the manufacturer's instructions.

RESULTS

Table 1 shows the results obtained with the two methods. Twelve and 11 of 21 MTB-positive cultures were also positive by Xpert® MTB/RIF ULTRA and Abbott RealTime MTB, respectively. Two additional positives of Abbott RealTime MTB were considered false positives, one of the controls and one of the MAC positive cultures. Compared to culture, the overall sensitivity and specificity for Xpert® MTB/RIF ULTRA were 57.1% and 100%, respectively, being 52.3% and 92.5% for the Abbott RealTime MTB. The differences were not statically significant. Of 21 samples positive for MTB, five were also positive by staining. These samples were among the positive NAAT results. There were no differences in the results according to the date of freezing.

DISCUSSION

In the present study, we analysed frozen aliquots remaining from decontaminated samples processed for mycobacterial culture to retrospectively diagnose lymphatic TB. The samples were analysed using two commercial NAAT [4,8], obtaining a sensitivity of 57.1% for Xpert® MTB/RIF Ultra and 52.3% for Abbott RealTime MTB. These results support the utility of a retrospective diagnosis for cases with clinical and/or histological suspicion of TB, days or weeks after processing a sample initially suspected of pathologies other than TB.

Both of the NAAT used in this study have been tested for diagnosing pulmonary TB. The new and old Xpert® versions, have been broadly tested [5,6], achieving a sensitivity of 89%

Table 1 **Xpert® MTB/RIF Ultra and Abbott RealTime MTB assays for the diagnosis of tuberculosis from frozen aliquots of lymph node samples processed for mycobacterial culture**

			Xpert® MTB/RIF Ultra		Abbott RealTime MTB	
Samples according to culture results	Combined results of staining and culture	Total number of samples	Positive	Negative	Positive	Negative
MTB	M+/C+	5	4	1	3	2
	M-/C+	16	8	8	8	8
Control	M-/C-	10	0	10	1	9
MAC	M+/C+	1	0	1	0	1
	M-/C+	6	0	6	1	5

M: microscopic examination; C: culture; MAC: *Mycobacterium avium-intracellulare* complex; MTB: *Mycobacterium tuberculosis* complex;

and a specificity of 99%. In addition, Abbott RealTime MTB has been used in several studies [8-10], showing a sensitivity of around 80%. Another study comparing both methods found equivalent results [11].

Several studies in the literature have described the results of NAAT in diagnosing lymphatic TB in fresh samples [4,7]. A Cochrane review [4] using Xpert® MTB/RIF Ultra reported a sensitivity of 70 to 81.6% with a specificity of 96.4-100%. Although the sensitivity results of the present study were lower than those achieved in fresh samples, they were comparable to other studies performed using paraffin-embedded biopsies. Moure et al [12] found a sensitivity of 60% and a specificity of 85.7%. Although the present study included samples frozen between 1 and 5 years previously, the results were independent of the freezing time. Among the few studies including frozen samples, some did not observe differences with respect to fresh samples [10,13]. It should however be noted that these studies included smear positive samples, with a high bacterial load. It has been suggested, that bacterial viability may be reduced in frozen samples due to autolysis of mycobacterial cells [14], and DNA degradation cannot be ruled out either. This reduction in viability would be evidenced only in paucibacillary samples, such as those from extrapulmonary sites, in which the bacterial load can be critical to decide a positive or negative result. Therefore, we hypothesized that freezing without measures to prevent bacterial autolysis could play a role in the decreased sensitivity found in the present study. On the other hand, processing frozen aliquots of remaining decontaminated culture samples not only from lymph nodes, can confirm a diagnosis of TB, weeks or months after obtaining the sample.

In conclusion, gene amplification performed in frozen samples allows confirmation of a diagnosis of TB weeks or months after obtaining the sample. For this purpose, both Xpert® MTB/RIF Ultra and Abbott MTB assays showed equivalent results.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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