ECRONICON OPEN ACCESS

EC PULMONOLOGY AND RESPIRATORY MEDICINE

Research Article

Sensitivity, Specificity and Predictive Values of PCR in Pleural Fluid in the Diagnosis of Pleural Tuberculosis

Jorge Chung Ching*

Pulmonologist, Hospital Nacional Arzobispo Loayza, Lima, Peru

***Corresponding Author:** Jorge Chung Ching, Pulmonologist, Hospital Nacional Arzobispo Loayza, Lima, Peru.
E-Mail: jorgechung14@gmail.com

Received: November 16, 2018; **Published:** December 26, 2018

Abstract

In order to determine the usefulness of real-time PCR in pleural fluid for diagnosis of pleural tuberculosis (TB PL) 404 cases of patients with pleural effusion in the Pneumology Service of Archbishop Loayza Hospital were selected between 2015 and 2016. Contingency tables were made and the values of sensitivity (S), Specificity (E), Positive predictive value (VPP) and Negative predictive Value (VPN) were found. For the study of Chi Square statistical significance was used.

Results: Of the 404 cases included in the study were diagnosed with Pleural TB 176. Only 66 cases had positive PCR. The findings were S: 34.8%, E: 98.2%, VPP 90.9% and VPN: 65.7%.

Conclusions: The real-time PCR is a test that must be taken as part of a battery of tests that provide a whole Pleural TB diagnosis but not shown to be useful as a rapid screening test and should not be used as a routine test.

Keywords: *Pleural Tuberculosis; Real-Time PCR; Study of Diagnostic Tests*

Abbreviations

TBC PL: Pleural Tuberculosis; rt-PCR: Real Time PCR; PCR: Polymerase Chain Reaction; ADA: Adenosine Deaminase; BX: Biopsia; S: Sensibilidad; E: Especificidad; VPP: Valor Predictivo Positivo; VPN: Valor Predictivo Negativo

Introduction

Tuberculosis remains the most prevalent infectious disease in the world. One of the main problems is the diagnosis that is often late and therefore the treatments are delayed causing a greater dissemination between contacts [1]. This problem is magnified in cases of extrapulmonary tuberculosis in which the characteristics of the bacillus and the difficult collection of samples for study can take weeks or months, and many of them remain hidden until after years [2-5].

In the case of pleural tuberculosis (TB PL), the diagnostic methods are far from optimal. Almost all are invasive and of low individual performance, with 80% being the total sensitivity [2,3]. To this must be added the time it takes to process the samples, at least 3 days for a biopsy, 30 days for a culture, and which can also be inconclusive, forcing a new procedure that is uncomfortable and bloody.

Against this background we can find a relatively rapid test based on molecular biology that amplifies specific DNA sequences allowing the identification of the mycobacterium even in very small quantities, so that it could be demonstrated in a short time [6-9]. To demonstrate that the Sensitivity (S), Specificity (E) and the Predictive Values both Negative (VPN) and Positive (VPP) are adequately high

in comparison with the current diagnostic methods, could lead us to select it as the test of choice in this pathology or otherwise discard it avoiding unnecessary costs [4,10,11].

Material and Methods

A descriptive, cross-sectional, retrospective observational trial was developed to correlate the diagnosis of TB PL by thoracentesis (Cytological, Biochemical, ADA Test), pleural biopsy (Pathology) and pleural biopsy culture with the study of pleural fluid by PCR for Mycobacterium tuberculosis in order to establish the usefulness of this diagnostic test.

The universe consisted of patients with Pleural effusion at the National Hospital Arzobispo Loayza during the years 2015 - 2016 who had biochemical, cytological, pleural biopsy and pleural biopsy culture in which PCR was also performed in real time in pleural fluid.

A patient with tuberculosis was considered to be the one who after the studies of Pleural Fluid (Cytological + Biochemical + ADA) + Culture for common fungi and germs Negative + Pleural Biopsy were included in the program to receive specific treatment. This result was compared with that obtained according to Real Time PCR of the Pleural Fluid in order to establish its usefulness.

Ethical aspects

Only patients who voluntarily agreed to the tests and completed the exams were included in the study. The signing of a consent document as part of the Informed Consent process of each patient with the attending physician was mandatory for the performance of the procedures of Thoracentesis and Percutaneous Pleural Biopsy. For this purpose, the consent forms valid in the Hospital were used.

Data processing

The data was collected in an instrument designed for the study and processed in SPSS 21.

Results

We included 404 patients who met defined criteria. The respective contingency tables were prepared for the study of diagnostic tests.

Table 1 shows the frequency distribution.

Diagnostic

		Frequency	Percentage	Valid Percentage	Accumulated percentage
Valid	Negative	226	55.9	55.9	55.9
	TBC PL	178	44.1	44.1	100
	Total	404	100	100	

rt-PCR

		Frequency	Percentage	Valid Percentage	Accumulated percentage
Valid	Negative	338	83.7	83.7	83.7
	Positive	66	16.3	16.3	100
	Total	404	100	100	

Biopsy

		Frequency	Percentage	Valid Percentage	Accumulated percentage
Valid	Negative	228	56.4	56.4	56.4
	Positive	176	43.6	43.6	100
	Total	404	100	100	

Cultivation of pleural biopsy

		Frequency	Percentage	Valid Percentage	Accumulated percentage
Valid	Negative	384	95.0	95.0	95.0
	Positive	20	5.0	5	100
	Total	404	100	100	

Table 1: *Frequency distribution.*

The prepared Contingency table shows the following distribution (Table 2).

Negative TBC PL			Diagnostic		Total
Rt-PCR	Negative	Count	222	116	338
		% within Diagnosis	98.2	65.2	83.7
	Positive	Count	4	62	66
		% within Diagnosis	1.8	34.8	16.3
Total		Count	226	178	404
% within Diagnosis		100	100	100	

Table 2: *Continuity table rt-PCR diagnostic.*

From this table the following is calculated: Sensitivity 34.8%; Specificity 98.2%; VPP 90.9%; VPN 65.7%.

It was determined that the contribution of the real-time PCR test in pleural fluid to the diagnosis of Tuberculosis is 1.8%.

For the calculation of statistical significance, the non-parametric CHI Square test was used, with the value of $p < 0.01$ as shown in table 3.

	Value	Degrees of Freedom	Significance Asymptotic Bilateral	Exact Significance Bilateral	Exact Significance Unilateral
Chi Square by Pearson	79.633	1	0		
Correction for continuity	77.233	1	0		
Likelihood ratio	89.410	1	0		
Fisher's Exact Test				0	0
N ° Valid cases	404				

Table 3: *Chi-square test.*

In addition, the S and E values of the PCR were calculated in real time against Pleural Biopsy and Biopsy Culture with their respective tests of statistical significance.

Negative TBC PL			Diagnostic		Total
Rt-PCR	Negative	Count	222	116	338
		% within Biopsy	97.4	65.9	83.7
	Positive	Count	6	60	66
		% within Biopsy	2.6	34.1	16.3
Total		Count	228	176	404
% within Biopsy		100	100	100	

Table 4: *PCR vs biopsia pleural.*

Chi square tests

	Value	Degrees of Freedom	Significance Asymptotic Bilateral	Exact Significance Bilateral	Exact Significance Unilateral
Chi Square by Pearson	71.923	1	0		
Correction for continuity	69.640	1	0		
Likelihood ratio	78.382	1	0		
Fisher's Exact Test				0	0
N ° Valid cases	404				

From this table the following is calculated: Sensitivity 34.1%; Specificity 97.4%.

The study found that the contribution of the real-time PCR test in pleural fluid to the diagnosis of Tuberculosis versus pleural biopsy is 2.6%.

Discussion

The diagnosis of diseases is mathematically a game of probabilities and the art of handling uncertainty. This applies perfectly to diagnostic tests [11,12]. Although the clinical history and physical examination are involved in the diagnostic process, special tests have been given special and vital importance since they provide us with sufficient evidence on which to base our diagnoses and treatments. A common problem in current clinical practice is to try to decide which test to select and when it is normal or pathological; and what this result means for the patient, above all [13,14].

Regarding TB PL, in many cases the clinical findings do not allow confirming or ruling out a diagnosis and it is important to estimate the likelihood that the clinical condition is caused or not by a tuberculous pathology. For this purpose, a series of available diagnostic tests are used, sometimes without estimating the validity of each one of them or their level of contribution to the diagnosis, thus misjudging its real significance [11,14,15].

The most important measures of the diagnostic value of a test are sensitivity (S) and specificity (E). These measure the diagnostic discrimination of the test compared to the reference criterion which, by definition, has an S and an E of 100% [14-17]. The S and the E represent intrinsic characteristics of a test that must be the same whether it is applied to a group of patients in whom the disease is rare or to a group of patients in whom it is frequent. For this reason, they provide measures of diagnostic discrimination, which must be the same regardless of the probability of illness before the test is performed [17-19]. The stability of the S and the E allows researchers from anywhere to apply the same diagnostic test and expect similar results despite the differences that exist between populations. These measures also allow researchers and clinicians to directly compare the performance of one test with that of others [18,19].

The S measures the proportion of individuals with the disease that are correctly identified by the test [20,21]. In the study, the value of this measure is in a very low range (34.8%) to be used to identify patients with Pleural TB since, in doing so, almost 65% of patients would not be diagnosed correctly and would be considered healthy.

On the other hand, the E measures the proportion of healthy individuals that are correctly identified as such by the test and in our study the value obtained is really high (98.2%). This value indicates that real-time PCR is able to identify with certainty patients who are not suffering from Pleural TB, which, however, should not lead us to think that it would be especially useful to rule out the disease.

The truth is that in diagnostic tests, just knowing the values of S and E usually does not have a real practical application since they are mathematically antagonistic and represent probabilities that become complicated when they do not have significant values [17,19,21,22]. This presents the problem of determining when a test with a positive or negative result corresponds to really sick or healthy subjects [22-24]. Therefore, we have determined the predictive values for this test that indicate the probability that the disease is present or absent after obtaining the results. These are strongly influenced by the prevalence of the disease in each place [4,20,22]. When the probability of a disease is moderately high before performing the test, for example 50%, even a negative test, leads to a probability that the disease is present. On the other hand, when the probability of the disease is relatively low before performing the test, for example, 10%, even a positive test leads to a probability that the disease is not present.

In the study, the calculation of the Positive Predictive Value (PPV) is 90.1%, which indicates that, in fact, real-time PCR is especially useful in identifying patients when we find a positive test, since only 10% turn out to be false positives. However, the Negative Predictive Value (NPV) is 68.7%, a very low figure to be able to consider CRP in real time as a useful discriminator in the identification of healthy subjects. This value would indicate that, in the face of a negative test, almost a third of them would actually be sick, diverting us from the diagnosis. The result is not consistent with the different studies published in other latitudes in which PCR is given values of VPN greater than 80%. In this regard, we must emphasize that these studies refer to populations with lower prevalence and with samples that are much smaller than those presented in this study.

In addition, the S and E values of the PCR against Pleural Biopsies have been calculated, being the similar values because in the great majority of cases the diagnosis is made by pathological anatomy. In a paper published in 2006 by Barrón., *et al.* the PCR was investigated against pleural biopsies embedded in paraffin, the results of the IS6110 TB-PCR test being: S of 96.7%, E of 100%, NPV of 94.7% and VPP of 100%.

The study found that the contribution of the real-time PCR test in pleural fluid to the diagnosis of Tuberculosis versus pleural biopsy is 2.6%.

When doing the respective calculation in front of Cultures of Pleural Biopsies the results are instead different. The S is 86.7% and the E 75%. The NPV rises 98.5% and the PPV drops to almost 23%. Although several publications mention that the culture of the pleural biopsies contributes almost 10% to the diagnosis, in this study it was determined that in our environment it only contributes 1.3% [25].

Conclusions

Real-time PCR is an emerging test that is becoming accessible to diagnostic services and has shown in different studies its great utility in the diagnosis of Tuberculosis[22]. In the case of TB PL, different biological specimens have been used, being of greater performance when performed in tissue from pleural biopsies.

According to what we found in the present study, we can determine that the real-time PCR test in Pleural Fluid despite having an E value close to 100% cannot be used as a rapid screening method for the diagnosis of pleural TB due to its low S and low VPN. Given its high PPV, it is especially useful in identifying patients when we find a positive test. It can be used in certain conditions to rule out this pathology or as part of a set of tests that in sum increase the probability of correctly identifying TB PL patients, but in our environment its use as a routine test is not justified.

Bibliography

1. Jimenez D., *et al*. "Diagnosis of pleural tuberculosis". *Chest* 121.3 (2001): 1005.
2. Melchor R and Yoldi M. "Papel actual de la biopsia transparietal en la patología pleural". *Revista de Patología Respiratoria* 2 (2001): 55-60.
3. Barrón H., *et al*. "Diagnóstico Molecular de Mycobacterium tuberculosis en biopsias pleurales embebidas en parafina". *Anales de la Facultad de Medicina* 67.1 (2006).
4. World Health Organization. "Global tuberculosis report 2013". WHO (2013).
5. Valdes L., *et al*. "Tuberculous pleural effusions". *European Journal of Internal Medicine* 14.2 (2003): 77-88.
6. Villegas M., *et al*. "Evaluation of polymerase chain reaction, adenosine deaminase and interferon gamma in pleural fluid for the differential diagnosis of pleural tuberculosis". *Chest* 118.5 (2000): 1355-1364.
7. Montenegro S., *et al*. "Improved Detection of Mycobacterium tuberculosis in Peruvian Children by use of a Heminested IS6110 Polymerase Chain Reaction Assay". *Clinical Infectious Diseases* 36.1 (2003): 16-23.
8. Light RW. "Update on tuberculous pleural effusion". *Respirology* 15.3 (2010): 451-458.
9. Escudero Bueno C., *et al*. "Cytologic and bacteriologic analysis of fluid and pleural biopsy specimens with Cope's needle. Study of 414 patients". *Archives of Internal Medicine* 150.6 (1990): 1190-1194.
10. Soini H and Musser JM. "Molecular diagnosis of mycobacteria". *Clinical Chemistry* 47.5 (2001): 809-814.
11. Catanzaro A., *et al*. "Rapid laboratory diagnostic tests for tuberculosis. What is the appropriate use?" *American Journal of Respiratory and Critical Care Medicine* 155.5 (1997): 1804-1814.
12. Gardiner DF and Beavis KG. "Laboratory diagnosis of mycobacterial infections". *Seminars in Respiratory Infections* 15.2 (2000): 132-143.
13. Navarro R Aldo., *et al*. "TBC en pediatría: Experiencia en biopsia pleural en un hospital de Lima - Perú". *Revista Peruana de Pediatría* 58.2 (2005): 40-43.
14. Dalovisio JR., *et al*. "Comparison of the amplified Mycobacterium tuberculosis (MTB) direct test, Amplicor MTB PCR, and IS6110-PCR for detection of MTB in respiratory specimens". *Clinical Infectious Diseases* 23.5 (1996): 1099-1106.

15. Aslanzadeh J., *et al*. "Comparison of culture and acid-fast bacilli stain to PCR for detection of Mycobacterium tuberculosis in clinical samples". *Molecular and Cellular Probes* 12.4 (1998): 207-211.

16. Beige J., *et al*. "Clinical evaluation of a Mycobacterium tuberculosis PCR assay". *Journal of Clinical Microbiology* 33.1 (1995): 90-95.

17. Suffys P., *et al*. "Evaluation of the polymerase reaction for the detection of Mycobacterium tuberculosis". *International Journal of Tuberculosis and Lung Disease* 4.2 (2000): 179-183.

18. Piersimoni C., *et al*. "Comparative evaluation of the new Gen-Probe Mycobacterium tuberculosis amplified direct test and the semi-automated Abbott LCx Mycobacterium tuberculosis assay for direct detection of Mycobacterium tuberculosis complex in respiratory and extrapulmonary specimens". *Journal of Clinical Microbiology* 36.12 (1998): 3601-3604.

19. Gomez-Pastrana D., *et al*. "Diagnosis of tuberculosis in children using a polymerase chain reaction". *Pediatric Pulmonology* 28.5 (1999): 344-351.

20. Delacourt C., *et al*. "Use of polymerase chain reaction for improved diagnosis of tuberculosis in children". *Journal of Pediatrics* 126 (1995): 703-709.

21. Cheng V., *et al*. "Clinical evaluation of the polymerase chain reaction for the rapid diagnosis of tuberculosis". *Clinical Pathology* 57.3 (2004): 281-285.

22. Gomez-Pastrana D., *et al*. "Comparison of Amplicor, in-house polymerase chain reaction, and conventional culture for the diagnosis of tuberculosis in children". *Clinical Infectious Diseases* 32.1 (2001): 3217-3222.

23. Barnes P. "Rapid diagnostic tests for tuberculosis: progress but not gold standard". *American Journal of Respiratory and Critical Care Medicine* 155.5 (1997): 1497-1498.

24. Montenegro-James S., *et al*. "Improved detection of Mycobacterium tuberculosis by nested PCR in paucibacillary specimens". *American Journal of Pathology* 153 (1998): 1658.

25. Fuentes Z and Garrido L. "Tuberculosis pleural: Estudio epidemiológico de 109 casos". *BSVM* 20.2 (2000).

Volume 8 Issue 1 January 2019