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Diagnostic analysis for TB: A comparative study between Bacilloscopy, Culture and PCR (Gene-Xpert) techniques

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Abstract— *The presented study aims to make a comparative analysis regarding the diagnosis of M. tuberculosis performed through Bacilloscopy, Culture and PCR exams held at the Clinical Pathology laboratory at HUGG - Rio de Janeiro/ Brazil from May to December 2015. Analyzing the effectiveness and efficiency of PCR, compared to Bacilloscopy and Culture exams. The results reveal a higher occurrence in males between 19 and 59 years of age. The results also show a sensitivity and specificity of the PCR, compared to bacilloscopy, 100% and 90% respectively and kappa index of 0.76. Regarding culture, Gold Standard, the PCR obtained a sensitivity of 100% and specificity of 93% and a kappa of 0.78, which shows a very good agreement between the exams and proves that the PCR technique is effective for the diagnosis of tuberculosis. The results of this study corroborate previous studies and provide important information for health managers, emphasizing the importance of agility in the diagnosis of tuberculosis for the treatment of patients with tuberculosis. It is recommended that more health units use this diagnostic method for the benefit of the patient who may already leave the health unit diagnosed and with treatment started.*

Keywords— ***Mycobacterium tuberculosis, PCR, Tuberculosis.***

I. INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases of humanity and it is a severy problem of Public Health, considered by WHO the most significant health problem worldwide.

Although Tuberculosis is a disease that has been recorded for six thousand years, only in the last fifty years has science been able to help patients concerning treatment. Six decades after the cure for the disease was found, tuberculosis still kills millions of people annually. (Kozakevich& Da Silva, 2016)

According to WHO in 2014, there were 9.6 million new TB cases, 5.4 million among men, 3.2 million women and 1 million children. There were also 1.5 million deaths from Tuberculosis (1.1 million were among HIV-negative and 0.4 million among HIV-positive), of which approximately 890,000 were men, 480,000 were women and 140,000 were children. The number of TB deaths is unacceptably high. With timely diagnosis and correct treatment, almost everyone with TB can be cured. (WHO, 2015).

WHO aims to develop the health of all people as much as possible.One of its actions is to monitor Tuberculosis around the world, as well as to disseminate recommendations for treatments and controls. (Procópio, 2014)

Brazil is one of the WHO member countries and cooperates with information related to public health. With this, the Ministry of Health tries to comply with all recommendations for diagnosis, treatment and control regarding Tuberculosis and other diseases of public health importance. (Marques, 2018)

From 2000 to 2014, WHO reported that 43 million lives were saved through effective diagnoses and treatments. (Roquete, 2017)

Tuberculosis is an infectious disease whose etiologic agent is the bacterium M. tuberculosis. Its infection occurs, most commonly, through the infected individual's cough, speech or sneeze. (Nogueira, 2012).

Each patient with pulmonary Tuberculosis that is not treated can infect an average of 10 to 15 people per year. Some factors contribute to the spread of the disease, such as poverty and poor income distribution, SIDA,

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malnutrition, poor sanitary conditions and high population density. (MinasGerais, 2006)

There are some tests for the diagnosis of Tuberculosis, but the most used are bacilloscopy and culture. According to ANVISA (2004), bacilloscopy consists of visualizing the bacteria through a microscope and the slide, which must be correctly stained. This consists of a quick procedure, but with risks of failure, due to human manipulation. Culture consists of a medium that favors the controlled growth of bacterial colonies and allowsvisualization, this procedure is more accurate than bacilloscopy.However, it is a very time-consuming procedure since the bacteria takes up to 60 days to grow.

Due to the possible failures and the long delay in the diagnosis of Tuberculosis and the fact that it is a highly infectious disease, there is a need to accelerate the diagnostic process.

According to Lira (2012), tuberculosis control consists of an early and effective diagnosis associated with adequate treatment, however, conventional methods have limitations, such as low sensitivity and late results. For this reason, molecular methods have been proposed for the diagnosis of several infectious diseases.

Pinhata (2014) corroborates that the rapid and accurate diagnosis is essential for tuberculosis control and that, to speed up the tuberculosis diagnosis process, the molecular method in PCR is recommended.

Because of the statements above, the present study aims to analyze the effectiveness and efficiency of the diagnoses of Mycobaterium tuberculosis obtained through PCR, bacilloscopy and culture in patients at the Hospital UniversitárioGaffréeGuinle – UNIRIO, in the city of Rio de Janeiro, belonging to the state of RJ - Brazil, from May 2015 to December 2015.

The present study hypothesizes that the PCR diagnostic method will be useful and agile in the diagnosis Tuberculosis, favoring the doctor who will get the diagnosis faster and the patient, if he is identified with positive tuberculosis, he can leave the hospital with the appropriate treatment.

II. METHOD

The presente study is aaplicate research, the aims is explicative and has a qualitative and quantitative approach. This research is also classified as prospective. Data were collected through biological samples from participating patients who were admitted to the Hospital UniversitárioGaffrée-Guinle – UNIRIO/ RJ, with suspected pulmonary tuberculosis who had a persistent cough of more than two weeks and who agreed to participate in the research by signing the informed consent form, from May to December 2015. The samples were subjected to laboratory tests such as PCR, bacilloscopy and culture and were performed in the Clinical Pathology laboratory of the Hospital UniversitárioGaffrée-Guinle.

The research project was submitted to analysis by the Research Ethics Committee of Universidade Salgado de Oliveira and approved under seem number 1184022.

The sample was of the probabilistic type and had 154 participants.

Biological samples of the following types were collected: sputum, lavage bronchus, urine, CSF, gastric lavage, lymph nodes and other tissues and sediments, in these volunteers. These samples follow to Clinical Pathology laboratory of the Hospital UniversitárioGaffrée-Guinle where they underwent three types of diagnostic analysis: bacilloscopy, culture and PCR.

In the bacilloscopy technique, the slide was prepared, and after preparation, the search for M. tuberculosis was done through visualization of the stained bacillus through an optical microscope.

In the Culture technique, the biological sample was fluidized; decontaminated and sown in solid egg-based culture medium (Löwenstein-Jensen)and stored at a temperature between 35 to 37 ° C for multiplication, in a period of up to 8 weeks.

In the PCR procedure, the biological sample was treated with the reagent for biological sample in the MTB-RIF kit suitable for the PCR (Genexpert).It was inserted into the cartridge, which also appears in the MTB-RIF kit and after this procedure the cartridge was placed in the PCR device (Genexpert) for reading that occurs within 2 hours.

The data obtained were tabulated using Microsoft Office Excel for Windows software version 2011, Graph Pad Prism software, and analyzed according to the three types of tests performed and the variables: sex, age, positive and negative for tuberculosis.

In the comparative analysis to evaluate the performance of the diagnostic techniques, the Qualitative Test was applied, which is used to know the quality of a diagnostic test and comprises the following items: Sensitivity, Specificity, Prevalence Positive Predictive Value, Negative Predictive Value and Kappa Index.

Kappa Index (k): it is used to verify the reliability of a test and through the k index, which is an advance in relation to the general agreement rate.It is an adjusted agreement indicator, as it takes into consideration, the due agreement the chance. The k reports the proportion of non-random

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agreement (in addition to that expected by the chance) between observers or measures of the same categorical variable, and its value ranges from "minus 1" (complete disagreement) to "plus 1" (total agreement). If the measure agrees more repeatedly than expected by the chance, then the k index is positive; if the agreement is complete, then k = 1. Zero indicates the same as readings taken at random. According to Landis and Koch (1977) the agreement between methods will be:

- Bad when the kappa index is less than 0.20,
- Weak when between 0.21 - 0.40,
- Moderate when between 0.41 - 0.60,
- Very good when between 0.61 - 0.80
- Excellent when greater than 0.80.

Parameters used to assess the diagnostic performance of the techniques (figure 1)

Sensitivity (S): is the probability of a test being positive in an infected or sick person, that is, it translates the percentage of infected or sick people correctly diagnosed by a positive test. The sensitivity corresponds to the proportion of the test's true positives: a / (a + b).

Specificity (E): it is the probability of a test being negative in an uninfected or non-sick person, that is, it translates the percentage of non-patients correctly identified by a negative test. Specificity corresponds to the proportion of the test's true negatives: d / (c + d).

Prevalence or Probability Pre-test: the proportion of sick individuals or the probability of individuals being sick, regardless of the test result: p = (a + c) / n.

Positive Predictive Value (PPV): it is the probability of an individual having the disease when the test is positive: a / (a + c).

Negative Predictive Value (VPN): it is the probability of an individual not having the disease when the test is negative: d / (b + d). The Predictive Value is determined by the sensitivity, specificity and prevalence of the disease in the tested population. The more sensitive the test, the better its negative predictive value. The more specific the test, the better its positive predictive value.

Diagnostic Efficacy, Accuracy or Precision: it is the test's ability to correctly classify the most significant number of individuals assessed as truly sick and healthy: (a + d / a + b + c + d).

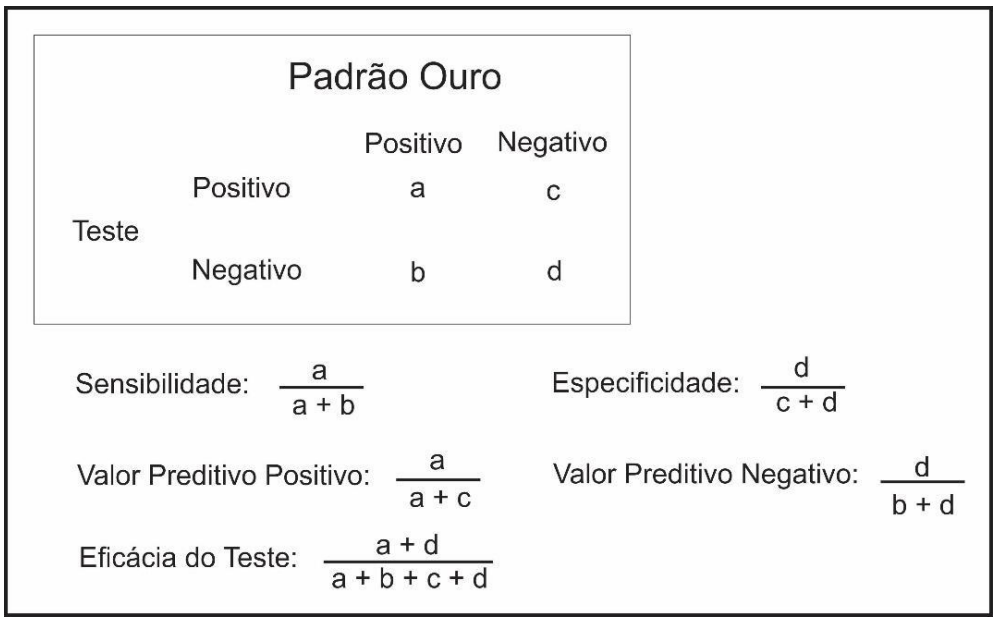

Fig. 1 - Parameters used to evaluate the performance of diagnostic methods.

Source: Landis e Koch, 1977

III. RESULTS

From the sample of 154 volunteers who underwent the three types of diagnostic tests (BAAR, Culture and PCR), it was possible to observe the following results:

3.1 The characteristics of the patients

In the study, it was verified the relation of the examinations performed with sex, where it was found that 70 individuals (45%) are female and 84 individuals (55%) are male.

These data corroborate with those found by Lira (2012) in his study on tuberculosis diagnosis with real-time CRP,

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who also observed a higher occurrence of suspected Tuberculosis in men, 58% and 42% in women.

Regarding age, one volunteer was in the age group up to 19 years old (child and youth), 101 volunteers were in the age group from 20 to 59 years old (adult) and 52 volunteers were in the age group older than 60 years old (older).

According to Lima et al (2008) the average age of the 160 patients in their study was 40.0 ± 12.8 years (range, 19-78 years).

According to Silva (2002), this frequency follows the national standart of disease incidence, that is, the most productive phase of the population.

3.2 Comparison between techniques and biological sample

The number of positive and negative tests was also observed according to the technique used. Where in the bacilloscopy technique, it was positive for 11 exams and negative for 143. In a Culture (gold standard) severalf 15 positive and 139 negative exams were observed, and in PCR, 24 exams were positive and 130 negative. (Table 1)

Table 1 - Tuberculosis diagnosis table according to the three techniques used.

	BAAR	CULTURA	PCR
SITIVE	11	15	24
GATIVE	143	139	130
TAL (n)	154	154	154

Source: author data

The type of biological sample (material collected) used for the diagnosis of Tuberculosis varied widely. It was used: Subcutaneous aspiration (12 samples), biopsy (2 samples) sputum (88 samples), bronchial lavage (34 samples), cerebrospinal fluid - CSF (10 samples), lymph node (3 samples) pleural fluid (5 samples), abdominal secretion (1 sample), tracheal secretion (5 samples), saliva swab (1 sample) and urine (4 samples).

According to the National Tuberculosis Control Program (2015) the recommended biological samples are sputum, induced sputum, bronchoalveolar lavage, CSF, lymph nodes, other tissues and gastric lavage.

Kox et al (1994) used a sputum blood sample, spinal brain fluid, pleural fluid, fistula fluid, pus and feces for the detection of M. tuberculosis DNA in patients with positive TB confirmed by culture. Moreover, they observed that DNA was amplified by the PCR technique, concluding that biological materials from different sites can be used in the PCR technique.

3.3 Results obtained by different tuberculosis diagnostic techniques

The rate of positivity obtained by reading the bacilloscopy technique was 7%, that is, 11 positive participants were detected for M. tuberculosis among the 154 individuals evaluated.

The positivity rate obtained by reading through culture was 10%, that is, 15 positive participants for M. tuberculosis were detected among the 154 individuals evaluated.

The rate of positivity obtained by reading through the PCR technique was 15%, that is, 24 positive participants for M. tuberculosis were detected among the 154 individuals evaluated.

In the evaluation of the results obtained by the 3 techniques (bacilloscopy, culture and PCR), of the 154 volunteers, 129 volunteers obtained the same negative result in the 3 techniques and 11 volunteers obtained the same positive results in the 3 techniques, with 14 volunteers who disagreed between the results as described in table 2 below.

Table 2 Comparison of the results of Bacilloscopy (BAAR), Culture (LJ) and PCR

Exame Results	**Total (n)**
BAAR (-), LJ (-),PCR (-)	129
BAAR (+), LJ (+), PCR (+)	11
BAAR (-), LJ (-), PCR (+)*	11
BAAR (-), LJ (+), PCR (+)	2
BAAR (-), LJ (+), PCR (-)	1
BAAR (+), LJ (-), PCR (+)	0
TOTAL	154

Source: author data

Confirmation of tuberculosis through diagnostic methods remains a significant challenge. According to Palomino (2005), the best method of diagnosing pulmonary TB is analysis based on a clinical, radiological and microbiological combination.

According to Redner (2015) when bacilloscopy and culture are negative and PCR positive, the sample may be of the paubacillary type.The patient may actually have TB or the M. tuberculosis bacillus is present, but this bacillus is "dead".

In this study, two patients were observed in which smear microscopy was negative and culture and PCR were

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positive. According to Redner (2015), this result is considered positive for TB.

It was also observed examination where bacilloscopy and PCR were negative, but the culture was positive. According to Redner (2015), this result is considered apaubacillary case or the mycobacterium is not part of the tuberculosis complex.

No case was observed in which bacilloscopy and PCR were positive, while culture was negative. Redner (2015) explains that this case can occur in positive cases for TB, paubacillary cases, or when there are M. tuberculosis bacilli, but these are dead.

3.4 Comparative evaluation between the results obtained by the bacilloscopy and PCR techniques

When comparing the results of the PCR test with the results obtained by bacilloscopy, it is observed that

thebacilloscopytechniquedidnotdetect 29 participantswith positive samplesby PCR.There was no positive bacilloscopy examination that was not negative by PCR. The positivity rates obtained by the techniques, 8.6% for bacilloscopyand 19% for PCR.

These proportions showed a statistically significant difference ($p < 0.0001$) *.

The Sensitivity observed in this comparison was 100% and the Specificity was 90%. Efficacy or Accuracy is 91%, VPP is 45% and VPN is 100% and Prevalence is 15%.

According to Chimara and Ferrazoli (2009), the reliability of the bacilloscopy reading dependens on the laboratory professional`sexperience, the quality of the biological sample, and the bacillary load present in the biological sample.

According to Redner (2015) when PCR results in a positive diagnosis and bacilloscopy results in a negative diagnosis (29 cases) the sample may contain mycobacteria, however, this may not be from the Tuberculosis Complex (MNT), the sample may be paubacillary or the patient has TB.

According to Soares (2011) the higher the sensitivity, the greater the VPN, that is, the greater the probability of having a negative result, there will be no disease.

3.5 - Comparative evaluation between the Culture and PCR techniques

The results of the PCR assay were compared with those obtained by the Culture technique. PCR was concordant in 15 positive samples by Culture. The PCR assay positivity rate was 19%, while the culture positivity rate was 11.5% ($p < 0.0001$) *.

A high sensitivity of 100% and specificity of 93% and Efficacy of 93% were also observed. The VPN was 100% and VPP was 60% and Prevalence was 16%.

According to Redner (2015) when the PCR results in a negative diagnosis and the culture results in a positive diagnosis (2 cases), the sample may be paubacillary or Mycobacteria may not be from the Tuberculosis Complex (MNT). Moreover, when PCR results in a positive diagnosis and Culture results in a negative diagnosis (23 cases) the sample may also be paubacillary or the bacillus is present, but “dead” or the patient does have TB.

According to Soares (2011) the greater the specificity, the greater the PPV, that is, the higher the probability of having a positive result of having a disease.

3.6 Evaluation of the performance of the PCR in comparison to the methods of Bacilloscopy and Culture.

The PCR performance was evaluated using the Bacilloscopy technique alone and the Culture technique alone. The PCR showed sensitivity of 100%, 90% ofSpecificity and Kappa Index of 0.76 when using Bacilloscopy as a comparison. When Culture was compared, the PCR obtained sensitivity of 100%, specificity of 93% and Kappa index of 0.78, which means that the agreement between the methods was excellent.

According to Soares (2011) the higher the prevalence, the higher the VPP and the lower the VPN, that is, the more frequent a disease is, the more likely it is to find true positives (increasing the value of the VPP), but it is also more likely to find false negative (decreasing the VPN). This is not the case in the present study.

According to Lima et al (2008) in their comparative research of Bacilloscopy with PCR, kappa of 0.54 and when compared to Culture with PCR, found a kappa of 0.78.

IV. CONCLUSION

The kappa index showed that the results found by the PCR are in good agreement with the results found by the culture, which leads to the conclusion that the methods are equivalent.

False-positive and false-negative results are generally associated with paubacillary biological samples, biological samples contaminated with other types of mycobacteria, but not of the TB complex or the presence of M. tuberculosis, however “dead”.

This study allowed us to conclude that the PCR, due to its high sensitivity has an excellent negative function for the exam.

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The culture is currently the gold standard test, but it takes 45 days to be ready, the bacilloscopy takes a few hours and the PCR, a maximum of two hours.

Based on this study, it is possible to say that the PCR presents the necessary conditions for the standardgold for the diagnosis of Tuberculosis.

This study allows to recommendthe competent authorities and the Ministry of Health, to make PCR as a standard test to identify tuberculosis.

However, further studies in this area and more significant acquisition of this method in other hospitals, in the coming years, can be configured as a benefit for both health managers and doctors and the population.

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