COMPARISON OF THE SENSITIVITY AND SPECIFICITY OF THE ZIEHL-NEELSEN METHOD WITH THE MOLECULAR RAPID TEST METHOD IN THE EXAMINATION OF BTA IN THE SPUTUM OF PATIENTS SUSPECTED OF PULMONARY TUBERCULOSIS

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ABSTRACT

Pulmonary tuberculosis or pulmonary TB is a disease that is easily transmitted through the air from the source of transmission, namely BTA positive TB patients when coughing or sneezing, patients spread germs into the air in the form of sputum splashes. For this reason, an examination method that has high sensitivity and specificity is needed as a tool for early detection of Pulmonary TB. **Research Objective**: To study the comparison of the sensitivity and specificity of the Ziehl-neelsen method and the Molecular Rapid Test method in examining BTA in the sputum of TB suspected patients at the Puskesmas against the gold standard using BTA culture.

This study is an analytic observational study with a cross sectional design. The population in this study were all patients suspected of having Pulmonary TB who came to the Kupang Health Center, Jetis Health Center, and Gedeg Health Center who performed microscopic and culture examinations in the laboratory during 2023. Sample selection used simple random sampling method with a sample size of 60 patients. The hypothesis was proven using the Kappa test.

Results: the sensitivity and specificity of the Ziehl-Neelsen method BTA examination were 60% and 100%, while the sensitivity and specificity of the Molecular Rapid Test method examination were 100% and 100%. The results of the Cohen's Kappa test showed that between the Ziehl-Neelsen and Molecular Rapid Test methods both had high sensitivity and specificity (Kappa; 0.733).

Conclusions and advice: Based on the results of the study, the molecular rapid test method has a higher sensitivity than the Ziehl-Neelsen method. This method can be recommended for early detection of pulmonary TB

Keywords: Pulmonary TB, Ziehl-Neelsen method, TCM method.

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INTRODUCTION

Tuberculosis (TB) is an infectious and contagious disease caused by Mycobacterium tuberculosis (MTB). TB is a disease that is easily transmitted through the air from the source of transmission, namely BTA positive TB patients when coughing or sneezing, patients spread germs into the air in the form of sputum splashes. One cough can produce about 3000 sputum splashes. Supporting examinations for TB disease can be carried out by examining molecular rapid tests, ziehl-neelsen BTA method examinations, thoracic photographs, tuberculin tests (mantoux) and MTB culture examinations. MGIT is a method for culture or growth of MTB. Lack of culture due to the nature of MTB slow at the time of division of about 20 hours, so the new growth dikultur appear after 4 - 8 weeks. (Nurul Husna, 2020).

The Molecular Rapid Test is a catridge test based on the Nucleic Acid Amplification Test (NAAT) that automatically detects TB cases and riampicin resistance, and can be performed even if the sputum sample is only 1 ml. The World Health Organization (WHO) recommends the use of TCM to evaluate TB suspect patients. TCM is considered to provide advantages for early diagnosis of TB and the use of this diagnostic system can increase the certainty of rapid diagnosis for all patients (Zuraida et al, 2021).

BTA examination Ziel-Neelsen method is used for bacterial identification (morphology / shape) requires a stain that uses predetermined dyes. Dyes that are

widely used include carbolic fuchsin, acid alcohol and methylen blue, so that baketri can be stained, previously a preparation must be made on a glass object (smear), where the smear is later dried at room temperature and the bacteria are fixed by heating over a flame. Early diagnosis of TB and detection of TB drug resistance improves survival because identifying it sooner will treat it at an earlier stage and reduce mortality (Kemenkes RI, 2012).

The Rapid Molecular Test (TCM) is an automated and integrated molecular test with Polymerase Chain Reaction (PCR) technique based on bacterial Deoxyribonucleic acid (DNA) testing to detect MTB and simultaneously detect the bacteria's resistance to rifampicin. TCM has a sensitivity of 96.5% in diagnosing Multi Drug Resistance Tuberculosis (MDR-TB) and a sensitivity of 96.1%. In detecting rifampicin resistance (Kurniawan E et al, 2016).

To diagnose a disease must have a good level of accuracy, so the validity of an examination method is needed to determine individuals who are sick and who are not sick (Komariah et al., 2022). The validity of an examination method can be done by assessing and specificity of the method (Siregar et al., 2018). Puskesmas Kupang Mojoketo district has a TB testing facility service with ZN and TCM methods. The examination used has good validity, so it is necessary to know the sensitivity and specificity values of the examination. This study will also test to determine the BTA examination of the Ziehl Neelsen method and the Molecular Rapid Test.

RESEARCH METHODS

This type of research is a quantitative analytical study that explains the sensitivity and specificity between examination variables with ziehl-neelsen staining and TCM examination in suspected pulmonary TB using a cross sectional approach. The sample was 60 sputum. Independent variables in this study are using the Ziehl-Neelsen method and the TCM method. The dependent variable in the study: sputum culture BTA diagnosis of TB enforcement (gold standard). This study was conducted in the work area of UPTD Puskesmas Kupang, Puskesmas Jetis, Puskesmas Gedeg. This study was conducted in September 2023. To determine the difference in sensitivity and specificity of the Ziehl-Neelsen method

with the TCM method, a statistical test was conducted with the Cohen's Kappa coefficient test. This test was conducted to determine the consistency between the two examination methods, namely the Ziehl-Neelsen method and the TCM method.

RESEARCH RESULTS

Table 1. Characteristics of respondents comparing the accuracy of BTA results of the Ziehl-neelsen method BTA strategy and the TCM method at the Puskesmas against the gold standard of using BTA culture.

Number	Characteristics	n	%
1	**Gender**		
	Male	28	46.7
	Female	32	53.3
2	**Age**		
	Young age	1	1.7
	Productive age	49	81.7
	Non-productive age	10	16.6
3	**Jobs**		
	Not working	1	1.7
	Housewife	25	41.7
	Farmer	11	18.3
	Private	17	28.3
	Retired	6	10.0
4	**Education**		
	Not yet in school	1	1.7
	Primary Education	34	56.7
	Secondary Education	23	38.3
	Higher Education	2	3.3
	Total	60	100

Based on Table 1. that of the 41 research respondents, the most female respondents were 32 people (53.3%) with a productive age range, almost all of them were 49 people (81.7%), the most private jobs were 17 people (28.3%) and most of the last education was elementary as many as 34 (56.1%).

Table 2. Frequency Distribution of comparison of the accuracy of BTA results of the Ziehl-neelsen method and the TCM method at the Puskesmas against the gold standard of using BTA culture

Number	Methods	F	%
	Ziehl-neelsen		
1	positive	3	5
2	negative	57	95
	TCM		
1	positive	5	8.33%
2	negative	57	91.67%
	Total	60	100

Table 2 shows that out of 60 research respondents underwent 2 BTA

examinations, namely the Ziehl-neelsen and TCM methods. Based on this table, it can be seen that the results of the examination with the TCM method show that there are 5 people (8.33%) who tested positive.

Table 3. Distribution of sensitivity and specificity analysis of BTA Ziehl-Neelsen Method

BTA Metode Ziehl-Neelsen	Culture (Gold Standart)		Total
	Positive	Negative	
Positive	3 (a) True Positive	0 (b) False Positive	3
Negative	2 (c) False Negative	55 (d) Trua Negative	57
Total	5	55	60

Based on Table 3, out of 60 research respondents who were examined for BTA Ziehl-Neelsen Method and compared positively with positive culture results as many as 3 people and BTA Ziehl-Neelsen Method negative and compared with negative culture results as many as 55 people. 2 people got false negatives. The sensitivity result is 60% and the specificity is 100%. It can be seen that the sensitivity of BTA Ziehl-Neelsen Method is 60 and the specificity is 100%.

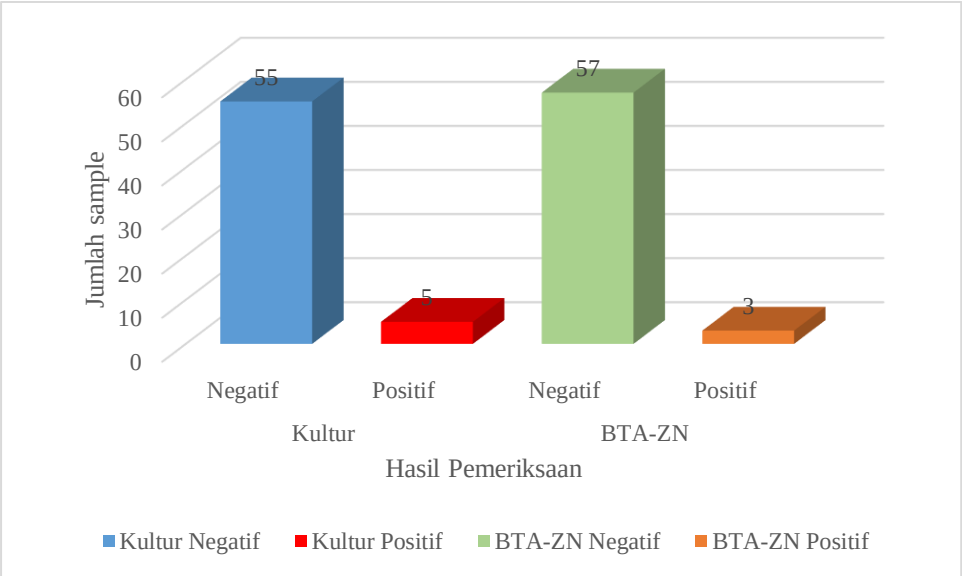

Figure 1. Comparison of the BTA Ziehl-Neelsen method and Culture Method.

Table 4. Distribution of sensitivity and specificity analysis TCM Method

TCM Methods	Culture (Gold Standart)		Total
	Positif	Negatif	
Postive	5 (a) True Positive	0 (b) False Positive	5
Negative	0 (c) False Negative	55 (d) True Negative	55
Total	5	55	60

Based on Table 4. It can be seen that out of 30 research respondents who were examined by TCM and compared with positive culture results as many as 5 people and negative TCM and compared with negative culture results as many as 55 people. The sensitivity result is 100% and the specificity is 100%.

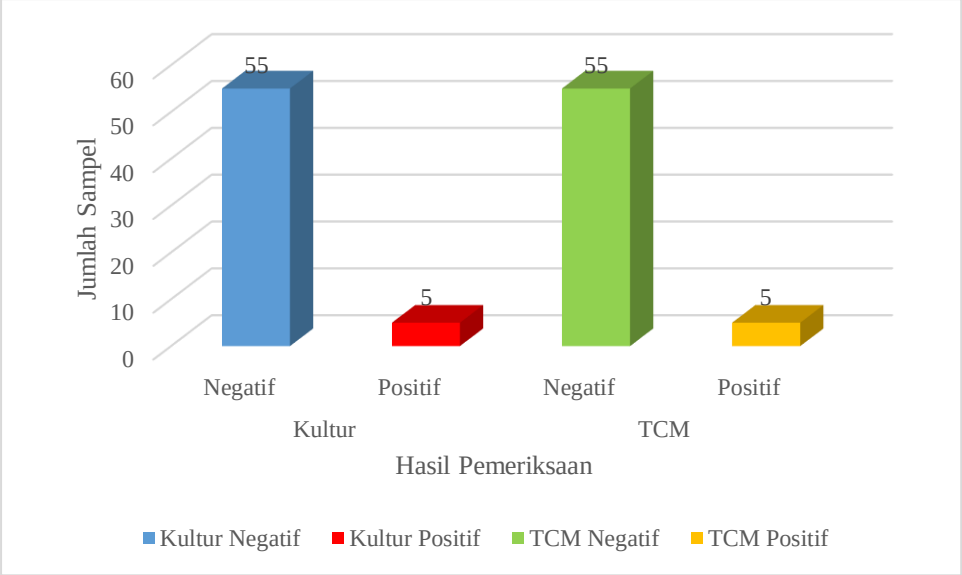

Figure 2. Comparison of TCM method and Kulure method

Table 5. Cross tabulation of the comparison of the accuracy of BTA results of the Ziehl-Neelsen method and the TCM method at the Puskesmas against the gold standard using BTA culture

Variable	Type of Lab Test		Total
	Positive	Negative	
Type of Lab Test	F	F	F
Ziehl-Neelsen	3	57	60
TCM	5	55	60
Total	8	112	120
P value Kappa = 0.733			

Based on Table 5. it can be seen that out of 60 research respondents who were

examined by TCM and compared positively with positive culture results as many as 3 people (5%) and those who were tested positive TCM and compared with positive culture results as many as 5 people. The Kappa test results between the Ziehl-Neelsen method compared to TCM were 0.733 (p value>0.05), meaning that the Ziehl-Neelsen method and the TCM method are equally sensitive compared to the gold standard.

DISCUSSION

1. **Sensitivity and specificity of Ziehl-Neelsen Method BTA to Culture Method BTA.**

   The results of research conducted with the Ziehl-Neelsen Method BTA examination showed positive results compared to positive culture results as many as 3 people (true positive) and negative Ziehl-Neelsen Method BTA compared to negative culture results as many as 55 people and 2 false negatives. The sensitivity result of BTA Ziehl-Neelsen Method is 60% and the specificity is 100%.

   The Ziehl-Neelsen (ZN) method and culture are the two main techniques used to detect Mycobacterium tuberculosis, the cause of tuberculosis (TB). Both have different advantages and disadvantages in terms of sensitivity, specificity and time. The Ziehl-Neelsen method is a staining technique used to detect acid-resistant bacilli (BTA) in sputum specimens. This method is simple and quick, with a positive result if there are more than 10,000 germs/ml of sputum. However, the sensitivity of this method is not always high, especially if the number of bacteria in the sputum is low. Research shows that the ZN method has a sensitivity that is not as high as its specificity, and positive results can be affected by the presence of non-tuberculosis bacteria (Evita et.al, 2019).

   In clinical practice at puskesmas Gedek in Mojokerto district, the choice between the Ziehl-Neelsen method and culture depends on the clinical context and available resources. The Ziehl-Neelsen method can be used for rapid diagnosis, while culture remains a more reliable method for confirmation and

determination of drug sensitivity. According to researchers, a combination of these two methods is often necessary to obtain an accurate and effective diagnosis in the management of tuberculosis. The results of research by nikmatul et.al, , 2020, Sensitivity and Specificity This Ziehl-Neelsen method has a lower sensitivity, with results showing a sensitivity of about 27% and specificity of 98% in some studies. This means that although positive results are very accurate, many TB cases can be missed (false negative results) if the number of bacteria in the sputum is low.The results of this study also obtained 2 false negatives, this is because the amount of sputum obtained from the patient was insufficient.Advantages of the Ziehl-Neelsen method Result Time: Results can be obtained quickly, usually within a few hours after sampling. Cost and Resources: This method is relatively inexpensive and does not require complex laboratory equipment, so it can be performed in many health facilities (Ariestoles et.al, 2024).

Researcher's opinion that the Ziehl-Neelsen method is suitable for rapid diagnosis, but has limitations in sensitivity, while the culture method is the gold standard in TB diagnosis, providing more accurate results despite requiring higher time and cost. A combination of these two methods is often required for comprehensive and accurate diagnosis in the management of tuberculosis.

2. Sensitivity and specificity of BTA TCM method against BTA culture method.

The results of the study carried out TCM examination and compared positively with positive culture results as many as 5 people (true positive) and TCM negative and compared with negative culture results as many as 55 people (true negative). In the TCM method, there were no false positives and false negatives. The sensitivity result is 100% and the specificity is 100%.

The TCM (Molecular Rapid Test) and culture methods are two techniques used to diagnose tuberculosis (TB) infection caused by Mycobacterium tuberculosis. Both have their own characteristics and advantages. TCM is a newer diagnostic method and has several advantages over traditional methods. The advantages of the TCM method, on sensitivity TCM has a sensitivity that varies between 73% to 93%, depending on the study

conducted. The average sensitivity is around 83%. Speed of Results: One of the main advantages of TCM is the faster time in providing results, which can be obtained in a matter of hours, compared to culture which takes longer, Resistance Detection: TCM not only detects the presence of TB bacteria, but can also identify resistance to rifampicin, one of the main drugs for TB, which is crucial in the management of therapy: The method can be used for all patients, both from the public and private sectors, and has been regulated in the national TB control program (Kurniawan, 2020).

Culture, especially using Lowenstein-Jensen (LJ) media, is the gold standard method in the diagnosis of TB. Gold Standard: Culture is considered the gold standard method for TB diagnosis. Although slower, culture provides highly accurate results in detecting Mycobacterium tuberculosis, Sensitivity and Specificity: The sensitivity of culture varies, but averages around 71% to 100% depending on the conditions and method used. Specificity is also high, reaching 90%, However, culture results can be affected by factors such as contamination and growth conditions. Process Time: The culture process takes a longer time, often up to several weeks, to obtain reliable results.

Although TCM showed higher sensitivity and better speed of results, there was no significant difference between the detection results of TCM and culture in some studies. The results of Ariestoles, 2024 showed a significance value (p) of 0.920, indicating that both methods have comparable performance in detecting TB infection Overall, both TCM and culture have important roles in TB diagnosis, and the choice of method can be adjusted according to clinical needs and the situation. TCM showed higher sensitivity in detecting TB infection. The average sensitivity of TCM ranges from 73% to 93%, while LJ culture has an average sensitivity of around 71%. The specificity of TCM is also quite good, with some studies showing rates above 80%.

LJ cultures have high specificity, but their sensitivity can be affected by factors such as contamination and growth conditions Response Time: TCM provides results in a much faster time, often within hours, compared to culture which can take up to several weeks to get accurate results. This allows for faster treatment of patients infected with TB, according to the researchers.A very

important advantage is that TCM can detect resistance to rifampicin, one of the main drugs for TB, immediately. This is very important in the management of TB therapy, especially in an era of increasing drug resistance.

3. **Comparison of sensitivity and specificity between TCM method and Ziehl Neelsen method in diagnosing tuberculosis.**

The results of the study conducted a positive TCM examination with positive culture results as many as 3 people (5%) and those conducted a positive TCM examination and positive culture results as many as 5 people. The result of the difference test between the BTA method compared to TCM is 0.717 (p value>0.05), meaning that the BTA lab test with the ZN method and the TCM Lab test are no difference, meaning that they are equally sensitive compared to the gold standard. The TCM method (GeneXpert) and the Ziehl Neelsen (ZN) method are two techniques used to detect Mycobacterium tuberculosis infection, the cause of tuberculosis. The comparison between the two methods is based on the effectiveness, sensitivity, and advantages and disadvantages of each.

If we compare the advantages of the Ziehl-Neelsen method include: (1) Speed: Results can be obtained in a short time. (2) Cost: Relatively cheap and does not require expensive equipment, (3) Simple: Easy procedure performed by trained medical personnel. The disadvantages are: (1) Low sensitivity: Especially in specimens with small numbers of bacteria. (2) Difficulty in interpretation: Positive results may be caused by other bacteria that are not pathogenic, (3) The results are often negative even if the patient is infected, with a positive detection rate of about 12.5%, (4) It takes longer to obtain results, and cannot detect resistance to drugs such as rifampicin.

TCM method: has higher sensitivity, with some studies showing positive results of up to 33% compared to 26% for Ziehl Neelsen can detect resistance to rifampicin simultaneously with bacterial identification, simultaneously with bacterial identification, TCM Disadvantages: Higher cost compared to the Ziehl Neelsen method, Requires specialized equipment and training to operate the GeneXpert device. Research shows that the TCM method has better sensitivity in detecting Mycobacterium tuberculosis. There are fewer true

negative results with the TCM method compared to Ziehl Neelsen, indicating that TCM is more effective in detecting infection.

It is the researchers' opinion that the TCM method (GeneXpert) is superior in terms of sensitivity and ability to detect drug resistance compared to the Ziehl Neelsen method. However, the cost and availability of tools are important factors in the selection of methods to be used in various health facilities. There were fewer true negative results with the TCM method compared to Ziehl Neelsen, suggesting that TCM is more effective in detecting infection, TCM also has higher specificity in detecting TB bacteria. BTA microscopic examination with Ziehl-Neelsen staining has a lower positive value compared to TCM. TCM can provide results in approximately 2 hours, which is faster than Ziehl-Neelsen staining which takes longer to provide results. TCM can give results in approximately 2 hours, which is faster than Ziehl-Neelsen staining which takes longer to give results, TCM can be used on a variety of specimens, such as sputum, gastric lavage, stool, etc., which allows TB testing in patients who cannot sputum directly. Thus, TCM offers several significant advantages in TB testing compared to Ziehl-Neelsen staining (Nikmatul, 2020).

The results of Karuniawan's research, 2020, three kinds of BTA staining methods, namely Tan Thiam Hok, Ziehl Neelsen, and Fluorochrome, were compared to the results of sputum culture on Lowenstein Jensen solid medium as a gold standard. Interpretation of staining results refers to the IUTLD scale. Mycobacterium tuberculosis growth was found in 27 out of 98 sputum specimens (27.6%) from 98 tuberculosis suspects. The sensitivity of Tan Thiam Hok, Ziehl Neelsen, and Fluorochrome staining methods were 62.9%, 81.5%, and 92.6%, while the specificity was 92.9%, 91.6%, and 91.1%, respectively. The positive predictive values were 77.3%, 78.6%, and 71.4%, respectively, while the negative predictive values were 86.8%, 92.9%, and 96.8%. From this study, it was found that TCM is the best method and can be done in a simple laboratory (Karuniawan, 2020). In contrast to this study which also differentiates with the TCM method. And the results are more effective TCM than the three methods above.

CONCLUSION

The sensitivity result of the Ziehl-Neelsen method was 60% and the specificity was 100%. The sensitivity result of TCM method is 100% and the specificity is 100%. The results of the Cohen's Kappa coefficient test between the Ziehl-Neelsen method compared to the TCM method were 0.733, meaning that the BTA microscopic test with the Ziehl-Neelsen method and the TCM method both have high sensitivity and specificity.

SUGGESTION

Based on the results of this study, the Puskemas can determine the use of the TCM method as an examination procedure to determine the diagnosis of Pulmonary TB. This research is expected to be a forum to increase knowledge and insight into examination methods that have high sensitivity and specificity for early detection of pulmonary TB.

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