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Clinical Evaluation of The Radiopharmaceutical ^{99m}Tc-Ethambutol to Diagnose Pulmonary and Extra-pulmonary Tuberculosis – Case Series

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ABSTRACT: Infectious tuberculosis disease is caused by Mycobacterium tuberculosis and can infect the lungs as well as other organs (extrapulmonary). Advanced diagnosis of TB disease using nuclear medicine methods involves the use of the radiopharmaceutical ^{99m}Tc-ethambutol. This study aims to clinically evaluate ^{99m}Tc-ethambutol for diagnosing both pulmonary and extrapulmonary TB. Research methodology is clinical scientific research based on the disciplines of nuclear medicine and nuclear pharmacy in marketing surveillance of new drugs. The research period was in 2023, conducted at multiple centers across three hospitals with a sample of 22 serial post-marketing surveillance cases. The examination results for TB in the subjects using the ethambutol patch test showed positive results in 40.9% and negative results in 59.1%. Comparative tests of the ethambutol patch test against treatment outcomes using X-ray methods yielded positive results in 40.9% and negative results in 59.1%, and using culture methods yielded positive results in 27.3% and negative results in 72.7%. The comparative results of TB examinations using the three methods showed the same results and were compared with treatment outcomes, with 6 subjects testing positive for TB, 13 subjects testing negative for TB, and 3 subjects with positive results being cases of extrapulmonary TB. All subjects diagnosed with TB underwent treatment. The measurements of the ethambutol patch test method using scintigraphy showed a sensitivity of 100%, specificity of 100%, and accuracy of 100%. The study concluded that the clinical evaluation of ^{99m}Tc-ethambutol can be used to diagnose both pulmonary and extrapulmonary TB. No drug side effects or subject complaints were found before or after the injection of ^{99m}Tc-ethambutol. Recommended to continue clinical evaluation with a larger number of subjects.

KEYWORDS: Clinical; diagnose; ^{99m}Tc-ethambutol; tuberculosis.

1. INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis (M.TB). M.TB is found and transmitted between humans via the airborne route. A large number of M.TB infections are often found to infect the lung parenchyma, causing pulmonary TB, but this bacterium also has the ability to infect other organs (extrapulmonary TB). Extrapulmonary TB includes organs such as the pleura, lymphadenopathy, urogenital tract, central nervous system and meninges, bones and joints, gastrointestinal tract, endometrium, pericardium, larynx, ears, and eyes [1]. The World Health Organization, in 2021, stated that Indonesia is the second largest country after India with the highest number of TB cases in the world [2]. The COVID-19 pandemic has hindered progress for years in providing TB services. Its impact is a significant global decrease in the number of people newly diagnosed and reported with TB, from 7.1 million in 2019 to 5.8 million in 2020, an 18% decrease to levels similar to those of 2012. Reduced access to TB diagnosis and treatment has led to increased deaths. The estimate in 2020 was 1.3 million TB deaths among HIV-negative individuals (up from 1.2 million in 2019) and an additional 214,000 among HIV-positive individuals (up from

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209,000 in 2019). This impact is expected to be much worse from 2021 to 2023. Patients with conditions resistant to anti-TB drugs (OAT), TB Multi-Drug Resistant (MDR), have a prevalence rate of 1.4% among new cases and 13.1% among old cases [3].

A radiopharmaceutical preparation, the ethambutol kit, has been discovered at the National Atomic Energy Agency to detect M.TB and dermatomusculoskeletal. The ethambutol kit received marketing authorization in 2020 from the Food and Drug Administration [4]. The ethambutol kit is labeled by direct labeling method using the metastable nuclear isomer of ^{99m}-technetium resulting in ^{99m}Tc-ethambutol [5]. ^{99m}Tc-ethambutol has underwent non-clinical testing to phase III clinical trials with the aim of detecting M.TB [6]. Radiopharmaceutical preparation of diagnostic kits based on the anti-tuberculosis drug, ethambutol [7]. In vitro and in vivo biological studies, as well as animal experiments, were conducted and the experiments showed ^{99m}Tc-ethambutol as a specific TB imaging agent. No adverse reactions were observed, it is safe for human use. ^{99m}Tc-ethambutol is localized in pulmonary and bone tuberculosis lesions [8]. ^{99m}Tc-ethambutol will accumulate at TB lesion sites and can be imaged using a gamma camera. The diagnostic value evaluation results of ^{99m}Tc-ethambutol scintigraphy in detecting and localizing TB in patients suspected of being infected with M.TB. ^{99m}Tc-ethambutol scintigraphy was visually analyzed, the results were compared with histopathology or microbiological tests [6].

In the clinical trial of the radiopharmaceutical ^{99m}Tc-ethambutol, based on the evaluation results as follows: Phase I clinical trial; an experimental study showing the potential of ^{99m}Tc-ethambutol as a TB imaging radiopharmaceutical. The number of subjects participating in the study is still limited (n=14 and 23) and not designed to compare head to head with standard TB diagnostic tests. The study included subjects diagnosed with pulmonary TB (n=4) and TB lesions in the bones (n=10) [8]. Phase II clinical trial; the clinical trial at this stage is also an experimental study showing the potential of ^{99m}Tc-ethambutol as a TB imaging radiopharmaceutical. The study included subjects diagnosed with pulmonary TB (n=7) and extrapulmonary TB with lesions in the bones (n=16). The study design is a prospective before and after design [6]. Phase III clinical trial; this clinical trial is a retrospective cross-sectional observational study aimed at evaluating the diagnostic utility of ^{99m}Tc-ethambutol scintigraphy in detecting and localizing TB infection. Subjects included in the study were subjects with histopathological or microbiological data diagnosed with TB infection, namely pulmonary TB (n=52), lymphadenitis TB (n=33), spondylitis TB (n=40), peritoneal TB (n=20), and other extrapulmonary TB (n=5). The study results showed a diagnostic concordance of ^{99m}Tc-ethambutol scintigraphy versus mycobacterial/histopathological of 92.9%.[6] This further study is a phase IV clinical trial (post-marketing surveillance) outlined in the results of this research in the form of case series [9]. This study objectives to clinically evaluate ^{99m}Tc-ethambutol for diagnosing both pulmonary and extrapulmonary TB.

2. MATERIALS AND METHODS

2.1. Material

The ethambutol kit contains a mixture of 3.5 mg ethambutol, 5 mg mannitol, 17.5 mg sodium pyrophosphate, and 1 mg $SnCl_2$ $2H_2O$ [10]. The quality testing of radiopharmaceuticals involved assessing the purity of the ethambutol kit labeling by Technetium pertecnetate solution from a ^{99m}Tc Generator. The labeling process of ^{99m}Tc-ethambutol involved mixing 1-2 ml of ^{99m}Tc solution with an activity of 10 mCi into an ethambutol kit, followed by shaking and incubating for 10 minutes at room temperature [11]. The purity level of ^{99m}Tc-ethambutol was analyzed using thin-layer chromatography (TLC) and paper chromatography with two solvent systems, namely paper chromatography with a stationary phase of Whatman-31 ET with 50% acetonitrile eluent and TLC using TLC silica with acetone mobile phase. The analysis results of the radiopharmaceutical purity of one ethambutol kit package obtained a radiochemical purity of ^{99m}Tc-ethambutol >90%. The Whatman chromatogram in acetonitrile showed two peaks: a peak of ^{99m}TcO2 at (Rf 0.0) and a peak of the mixture (^{99m}TcO4 + ^{99m}Tc-ethambutol) at (Rf 8-10), with ^{99m}TcO2 obtained at 3.33%. The silica chromatogram in acetone also showed two peaks: a peak of the mixture (^{99m}TcO2 + ^{99m}Tc-ethambutol) at (Rf 0.0) and a peak of TcO4 at (Rf 8-10), with TcO4 obtained at 4.27%. The percentage purity of ^{99m}Tc-ethambutol met the radiopharmaceutical requirements, i.e., >85%.[5] The pharmacokinetic parameters of the radiopharmaceutical ^{99m}Tc-ethambutol have been previously studied, as outlined in Table 1 below.

Table 1. Pharmacokinetic Parameters of Radiopharmaceutical ^{99m}Tc-ethambutol [8].

No.	Parameter	Characteristics
1	Injection	intraveneous
2	Biodistribution	1 h (kindey>liver>intestines>blood)
		4 h (kindey>liver>intestines>stomach)
3	Half life	9.5 h
4	Volume of distribution	2 8 L/Kg
5	Clerance	16.5 mL/h
6	Plasma binding	60%
7	Excretion	intiniated by kidneys, then hepatobiliary system
8	Dosage	10-30 mCi
9	Chemical Properties	Ph 6.0-6.5
		Radiochemical purify 90%
		negative electric charge

2.2. Method

Research methodology is clinical scientific research based on the disciplines of nuclear medicine and nuclear pharmacy in marketing surveillance of new drugs which will be applied to health service policies. This study was conducted in a prospective observational manner on subjects with a clinical diagnosis of TB who were referred for ethambutol testing at a hospital that has nuclear medicine facilities. The research period was in 2023, conducted at multiple centers across three hospitals with a sample of 22 serial post-marketing surveillance cases.

The research subyek criteria are: a) inclusion criteria: suspected TB patients or TB patients who are referred for diagnostics to Nuclear Medicine, carry out a complete blood laboratory examination 1 week before and after a TB full body scan (maximum 1 week before/after), and diagnostic M.TB examination using other methods (rongent thorax/lumbo sacral x-ray and culture); and b) exclusive criteria: pregnant or breastfeeding patients, and patients under 5 years old.

Clinical evaluation of the radiopharmaceutical ^{99m}Tc-ethambutol was conducted in the following stages:

a. Preparation procedure of ethambutol dry kit
The ethambutol kit is packaged in primary vials and secondary boxes (containing 5 vials per box). The characteristics of the ethambutol kit, in the form of dry powder, appear good, sterile, and stable for a long period (storage for 8 months at a temperature of 4°C), as well as during transportation (5 hours at room temperature). After labeling the ethambutol kit with the radionuclide Technetium-^{99m}, it provides high radiokinetic purity (>90%) [5].
b. Procedure for preparing injectable radiopharmaceuticals
The preparation procedure involved adding the radioisotope ^{99m}-Tc to the ethambutol kit in amounts of 10-20 mCi (1-2 ml). It was then stirred and allowed to react for 5-10 minutes at room temperature. The resulting preparation is the injectable radiopharmaceutical ^{99m}Tc-ethambutol [5]. The radioisotope was obtained from a mini-generator in the nuclear medicine facility of the hospital [12].
c. Procedure for injecting radiopharmaceuticals into subjects
The standard operating procedure for injection involved the process of introducing fluid into the body using a syringe by a nurse. The injection was administered intravenously, and the fluid was directly introduced into the bloodstream. Before and after injection with ^{99m}Tc-ethambutol, complete blood examinations were conducted in the hospital laboratory, and the patient's condition was examined by a specialist in nuclear medicine [6].
d. Procedure for ethambutol scan
The standard operating procedure for the ethambutol scan involves imaging using a gamma camera conducted at 1 hour and 4 hours after the subject was injected with the radiopharmaceutical. After the injection, they wait for 1 hour in the radiation isolation waiting room. At exactly 1 hour, the subject underwent planar imaging with a gamma camera and SPECT/CT for approximately 10 minutes. Upon completion, the subject was asked to wait again in the radiation isolation waiting room until 4 hours after being injected with the radiopharmaceutical. At exactly 4 hours, the subject underwent

planar imaging again with a gamma camera and SPECT/CT for approximately 10 minutes. The interpretation of the imaging results was performed by a specialist in nuclear medicine [6].

e. Procedure for blood laboratory examination
The standard procedure for a complete blood examination and peripheral blood examination for all subjects performed before ethambutol scanning (up to 5 days) and after ethambutol scanning (1-2 days later). The blood examination results were interpreted by a clinical pathologist [6].

f. Procedure for monitoring the condition and complaints of subjects
The standard procedure for monitoring the condition and complaints of subjects before and after the injection of the ^{99m}Tc-ethambutol was conducted by a specialist in nuclear medicine. The parameters monitored include the presence of complaints such as upper abdominal pain, nausea, vomiting, dizziness, joint pain, headache, fatigue, dry throat/mouth, and red/itchy skin [6].

g. Procedure for comparing TB examinations
The procedure for comparing TB examinations was conducted in two ways: radiological examination with thoracic/lumbosacral X-ray and culture/bacterial cultivation examinations in the laboratory. Radiological examination uses X-ray photos, and the examination results were interpreted by a specialist radiologist. Culture examination was performed using culture media such as egg-based media, Lowenstein-Jensen, or agar media such as Middle-Brook, and the examination results were interpreted by a specialist in clinical microbiology. This examination is the gold standard in diagnosing pulmonary TB [6].

3. RESULTS

The results of the study on the clinical evaluation of ^{99m}Tc-ethambutol using nuclear medicine methods in diagnosing pulmonary TB and extrapulmonary TB are described. The research was conducted at three hospitals: Hasan Sadikin Hospital, Bandung, Gatot Soebroto Hospital, Jakarta, and Dharmais Cancer Hospital, Jakarta. The data were collected during the period 2023 with a total of 22 subjects, who were serial cases from post-marketing surveillance examinations conducted by the researchers. The data obtained are presented in narrative form and tables.

Table 2. Distribution of Subject Characteristics and TB Examination Results.

No.	Characteristics	Number	Percentage
1	Age		
	11-19 years (adolescents)	2	9.1%
	20-44 years (adult)	8	36.4%
	45-59 years (pre-elderly)	8	36.4%
	>59 years (elderly)	4	18.2%
	Total	22	100%
2	Gender		
	Male	14	63.6%
	Female	8	36.4%
	Total	22	100%
3	Ethambutol Test Method		
	Positive	9	40.9%
	Negative	13	59.1%
	Total	22	100%
4	Thoraric / Lumbosacral X=ray Method		
	Positive	9	40.9%
	Negative	13	59.1%
	Total	22	100%
5	Culture Method		
	Positive	6	27.3%
	Negative	16	72.7%
	Total	22	100%

Based on Table 2, the proportion of male subjects is higher than female subjects, with a predominance of adult and pre-elderly age groups compared to adolescents. Elderly subjects were also found in this study, totaling 4 subjects. The TB examination results of subjects using the ethambutol test method, thoracic/lumbosacral X-ray, and culture showed no missing data, with a complete total of 22 data [14].

The dose of ^{99m}Tc-ethambutol received by all subjects ranged from a minimum of 11.68 mCi to a maximum of 15.60 mCi. Previous studies stated that the safe and effective radioisotope dose range for humans is 10-30 mCi. [8] Using a dose in this range provides optimal imaging by a gamma camera.

Table 3. Distribution of Types of Pulmonary TB and/or Extrapulmonary TB Cases.

No.	Characteristics	Number
1	TB Pulmonary	6
2	TB Extrapulmonary (spondilitis TB osilium dextra)	1
3	TB Ektra Paru (Limfadentis TB)	1
4	TB Pulmonary and TB Extraoulmonary (spondilitis TB letion area bone vertebrata C5 Th 1 and apikal pulmonary sinistra)	1
	Total	9

Based on the Table 3, the results of TB examination using the ethambutol fingerprint method are: positive for 9 subjects and negative for 13 subjects. Positive examination results from ethambutol fingerprints consisted of: 6 cases of pulmonary TB and 3 cases of extra-pulmonary TB. In the ethambutol examination results, 3 cases of extra pulmonary TB could not be detected using the culture method. M. TB.

Two subjects with cases of pulmonary TB and extrapulmonary TB were described. Case 1 was a male, aged 60 years, with an initial indication of suspected pulmonary TB. Ethambutol test procedure, planar whole body scan imaging, and thoracic SPECT/CT were performed at 1 hour and 3 hours post-intravenous injection of radiopharmaceuticals. Imaging results from static whole body and SPECT/CT localization images showed pathological radioactivity uptake in the right apical lung mass and consolidation around the medial lobe cavity of the right lung at 1-hour images, which increased at 3-hour images. No increased pathological radioactivity uptake was observed in other organs. Physiological radioactivity uptake was observed in the mediastinum, heart, liver, spleen, kidneys, and urinary bladder. The subject's diagnosis was active infection, M.TB in the lungs.

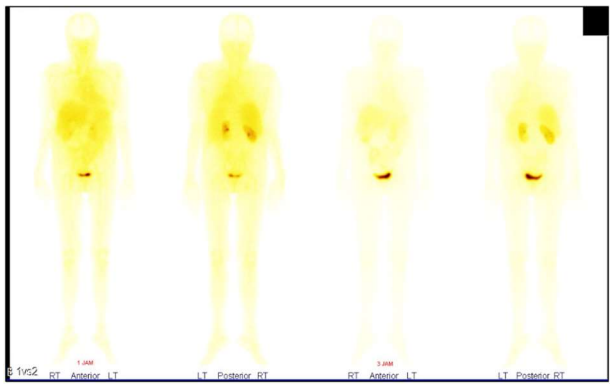

Figure 1. Results of full-body scan at 1 hour and 3 hours.

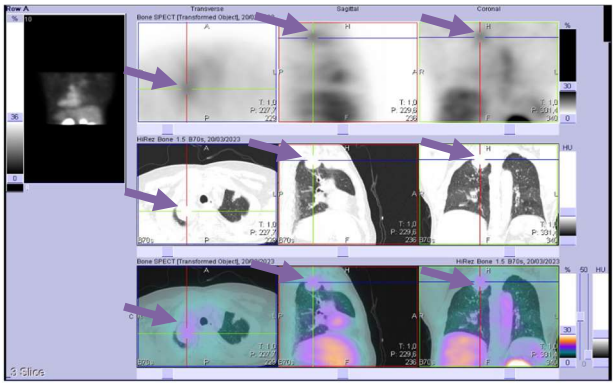

Figure 2. SPECT/CT Results.

Figure 1 shows the results of ethambutol test presented in whole-body planar imaging. Pathological radioactivity uptake is observed in the parenchyma of the right apical lung. Figure 2 shows the results of ethambutol test presented in SPECT/CT imaging of the lungs. As indicated by the orange arrows, the location of M.TB detected in the right apical lung and consolidation around the medial lobe cavity of the right lung.

Case 2 was a female, aged 37 years, with an initial indication of evaluating TB infection in a patient with a history of suspected TB lymphadenitis. Ethambutol test procedure, whole-body planar imaging with spots in the neck region, and lungs were performed at 1 hour and 4 hours post-intravenous injection of radiopharmaceuticals. Imaging results showed pathological radioactivity uptake persisting over time in the projection of the right cervical lymph nodes, while radioactivity uptake in other parts of the body remained within normal limits. The subject's diagnosis was consistent with TB lymphadenitis.

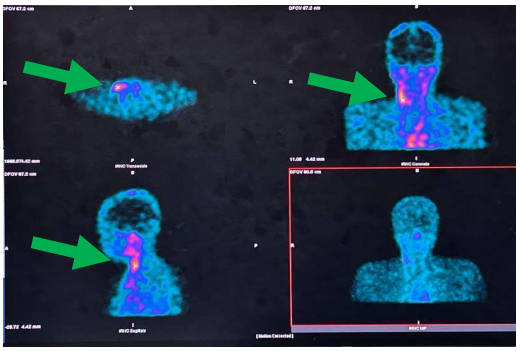

Figure 3. Results of static scan of neck region at 1 hour.

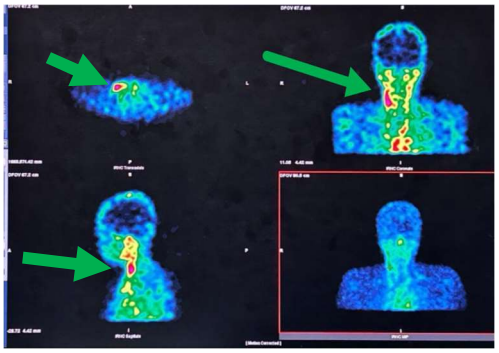

Figure 4. Results of static scan of neck region at 4 hours.

Figures 3 and 4 show the results of ethambutol test presented in static SPECT/CT imaging of the head and neck region. As indicated by the green arrows, the location of M.TB in the subject's lymph nodes. Clear uptake of radioactivity persisting pathologically in the lymph nodes of the right neck is observed. This is also supported by the palpation results of the subject by the nuclear medicine specialist, showing a lump in the lymph nodes on the right side before the subject underwent ethambutol test.

Cross-tabulation analysis of TB examination results with treatment actions can be seen in Table 4. Positive results for pulmonary TB were found in 6 subjects and positive results for extrapulmonary TB were found in 3 subjects. Negative TB results were found in 13 subjects.

Table 4. Cross-tabulation Summary of TB Examination Methods with Treatment Actions.

No.	Qualification/Distribution	Thoracic/Lumbosacral X-ray		Culture		Ethambuthol Test	
		Number	%	Number	%	Number	%
1	Positive	9	40.9	6	27.2	9	40.9
2	Negative	13	59.1	16	72.2	13	59.1
3	Missing value	0	0.0	0	0.0	0	0.0
4	Total	22	100.0	22	100.0	22	100.0

Cross-tabulation analysis describes the cross-tabulation results for each method (ethambutol test, thoracic/lumbosacral X-ray, and culture) with treatment actions.

Table 5. Cross-tabulation of TB Examination Results with Treatment Actions [14]

Crosstab			Post-Treatment Action		Total
			Treated	Not Treated	
Ethambutol Test	Positive	Count	9	**0**	**9**
		Expected Count	3.7	5.3	9
		% within Ethambutol Test	100.00%	0.00%	100.00%
	Negative	Count	0	13	13
		Expected Count	5.3	7.7	13
		% within Ethambutol Test	0%	100%	100.00%
Total		Count	9	13%	22
		Expected Count	9	13%	13
		% within Ethambutol Test	40.90%	59.10%	100.00%
Ethambutol Test	Positive	Count	9	**0**	**9**
		Expected Count	3.7	5.3	9
		% within Rontgen	100.00%	0.00%	100.00%
	Negative	Count	0	13	13
		Expected Count	5.3	7.7	13
		% within Rontgen	0%	100%	100.00%
Total		Count	9	13	22
		Expected Count	9	13	22
		% within Rontgen	40.90%	59.10%	100.00%
Ethambutol Test	Positive	Count	6	0	6
		Expected Count	2.5	3.5	6
		% within Rontgen	100.00%	0.00%	100.00%
	Negative	Count	3	13	16
		Expected Count	6.5	9.5	16
		% within Culture	18.70%	81.30%	100.00%
Total		Count	9	13	22
		Expected Count	9	13	22
		% within Culture	40.90%	59.10%	100.00%

Statistical tests were conducted to test the relationship between each TB examination method (ethambutol test, thoracic/lumbosacral X-ray, and culture) and treatment actions. *Chi-square* tests were performed individually for each examination method. The relationship between TB examination methods and post-treatment actions can be observed through the *p-value (asymptotic significance)* < *alpha = 0.01* or *p-value Exact Sig. (2-sided)* < *alpha = 0.01*. Additionally, to observe the strength of the relationship (correlation), the *phi coefficient* value can be examined since the data used are nominal and in a 2x2 format. [14] *Chi-square* testing, *phi coefficient* correlation values were calculated using SPSS Statistics and the results are presented in Table 6.

Table 6. Test Results of *Chi-square* and *Phi Coefficient.*

Testing method	Exact sig (2-sided)	Phicoefficient	Interpretation of Correlation Coefficient
Thoraric/lumbosacral X-ray *Treatment action	0.000	1.000	Perfect Correlation
Culture *Treatment action	0.001	0.736	High correlation
Ethambutol Test *Treatment action	0.000	1.000	Perfect correlation

The *chi-square* and *phi coefficient* test for ethambutol skin test showed a relationship between the ethambutol skin test method and post-treatment actions. The correlation value between them was 1.00, indicating a perfect correlation. The *chi-square* and *phi coefficient* test for thoracic/lumbosacral X-ray showed a relationship between the thoracic/lumbosacral X-ray method and treatment. The correlation value between them was 1.00, indicating a perfect correlation. The *chi-square* and *phi coefficient* test for culture showed a relationship between the culture testing method and post-treatment actions. This is supported by the correlation value between them, which was 0.736, indicating a high correlation.

Accuracy measurements for the ethambutol test method, TP (true positive) and TN (true negative) values indicate the level of classification accuracy. Generally, the higher the TP and TN values, the better the classification accuracy. If the predicted output label is true and the actual value is false, it is called FP (false positive), while if the predicted output label is false and the actual value is true, it is called FN (false negative).[16] The formula for calculating accuracy is as follows:

$$Accuracy = \left[\frac{a+d}{n}\right] \times 100\% \qquad Sensitivity = \left[\frac{a}{a+c}\right] \times 100\% \qquad Specificity = \left[\frac{d}{b+d}\right] \times 100\%$$

The results of ethambutol skin test scintigraphy showed sensitivity of 100%, specificity of 100%, and accuracy of 100%. The higher the sensitivity value of the examination method, the better the method is at detecting actual patient cases, thereby reducing the risk of false negative results. The higher the specificity value of the examination method, the better the method is at identifying truly negative patients, thereby reducing the risk of false positive results. Thus, specificity measures how well the examination method can correctly distinguish between healthy cases. The higher the accuracy value of the examination method, the better the method is at correctly distinguishing between the two types of cases, in this case positive or negative TB, thereby reducing the risk of incorrect results.

Complete blood examination results from all subjects showed normal results, both before and after injection of ^{99m}Tc-ethambutol radiopharmaceuticals. The hematological parameters measured were hematology (hemoglobin, hematocrit, erythrocytes, platelets, leukocytes, MCV, MCH, and MCHC), SGOT, SGOT, urea, creatinine, peripheral blood morphology, before and after injection of ^{99m}Tc-ethambutol radiopharmaceuticals, no abnormal data were found from the standard blood reference values. Reference values: hematology: hemoglobin 12.5-17.3 g/dL (male) and 11.8-15.4 g/dL (female); hematocrit 38.1-50.4% (male) and 31.4-49.7% (female); erythrocytes 4.2-6.2 million/ul; platelets 150,000-500,000/ul; leukocytes 4,400-10,000/ul; MCV 80.1-94.3 fL, MCH 25.9-31.9 pg; MCHC 31.4-35.2 g/dL; SGOT: 5-40 u/L and SGPT 7-56 u/L; urea: 14-39 mg/dL (male) and 12-33 mg/dL (female); creatinine: 0.64-1.36 mg/dL; peripheral blood morphology: basophil 0-1; eosinophil 1-3; band neutrophil 2-6; segmented neutrophil 50-70; lymphocyte 20-40; monocyte 2-8 [6]. Monitoring of drug side effects based on complete blood examination results showed no drug side effects before and after subjects were injected with ^{99m}Tc-ethambutol radiopharmaceuticals.

Monitoring of complaints from all subjects showed normal results without complaints, both before and after injection of ^{99m}Tc-ethambutol radiopharmaceuticals. The monitored complaint parameters were upper abdominal pain, nausea, vomiting, dizziness, joint pain, headache, fatigue, dry throat/mouth, and red/itchy skin [6]. No subjects were found with complaints before or after injection of ^{99m}Tc-ethambutol radiopharmaceuticals.

4. DISCUSSION

Referring to the results of this study on the clinical evaluation of ^{99m}Tc-ethambutol using nuclear medicine methods in diagnosing pulmonary TB and extrapulmonary TB, the discussion is elaborated as presented. One of the novel elements of this research is the multi-center study location, namely: three hospitals equipped with nuclear medicine facilities. Another new finding is that the primary data presented in this study are serial cases in the first post-marketing surveillance clinical trial in Indonesia conducted by the researchers with the support of many parties.

The characteristics of the subjects' gender, namely: the number of males (14 subjects) is greater than females (8 subjects). The comparison of genders occurs according to the patient number sequence in the study. The sampling method used was random sampling, following previous research, which stated that research samples could be obtained from randomly selected subjects studied at the research site.[18] The subjects' age characteristics are as follows: the number of adolescent subjects is 2, the number of adult subjects is 8, the number of pre-elderly subjects is 8, and the number of elderly subjects is 4. Age grouping is in accordance

with health field regulations.[1] It can be stated that the age group distribution represents a representative sample, and there are also elderly subjects in the number of 4, which can serve as supporting data for the safety level of using ^{99m}Tc-ethambutol in individuals above 59 years old.

The results of TB examination using the ethambutol spot method are as follows: positive for 9 subjects and negative for 13 subjects. The results of TB examination using thoracic/lumbosacral X-ray are: positive for 9 subjects and negative for 13 subjects. The results of TB examination using culture method are: positive for 6 subjects and negative for 16 subjects. Positive results from ethambutol spot examination consist of: 6 cases of pulmonary TB and 3 cases of extrapulmonary TB. In the ethambutol spot examination results of 3 cases of extrapulmonary TB, TB cases were not detected by the culture examination method. M.TB in cases of extrapulmonary TB was not present in the subjects' sputum, so during the culture examination, no M.TB was found.

The administered doses of ^{99m}Tc-ethambutol received by all subjects ranged from a minimum of 11.68 mCi to a maximum of 15.60 mCi. Previous studies have mentioned that the safe range for radioisotope dosage is 10-30 mCi [8]. Another study indicated that the use of ^{99m}Tc-ethambutol within the safe dose range is safe for pediatric patients.[6] Regarding the biodistribution of radiopharmaceuticals based on previous research, it has been stated that the radioisotope ^{99m}Tc is the most commonly used and safe radiopharmaceutical for hospital patients.[19] It can be stated that all doses of ^{99m}Tc-ethambutol used in this study fall within the safe dose range. The quality of the radiopharmaceutical is indicated by the purity values in the Whatman chromatogram test, with a purity level of 92.40%, and a minimum value of 90.0%. According to previous studies, radiopharmaceutical purity is >85% [16]. Radiopharmaceutical purity significantly affects the diagnostic results. If radiopharmaceutical purity falls below the established acceptability standard, the radiopharmaceutical will not target organs infected with M.Tb bacteria. Administration of the ethambutol radiopharmaceutical to patients is done via intravenous injection. Ethambutol will deliver the Technetium-^{99m} radioisotope (140 keV gamma energy) to organs infected with M.TB bacteria. After reaching the target, patients are scanned using single-photon emission computed tomography (SPECT) gamma cameras. Overall, the ^{99m}Tc-ethambutol radiopharmaceutical has a bioavailability of 100% when administered via intravenous injection [20]. It can be stated that the quality of the ^{99m}Tc-ethambutol radiopharmaceutical in this study meets the purity requirements for medical injection into patients.

Based on the results of research on the diagnose of TB cases in table 3, there were 2 cases: case 1 involved a subject diagnosed with pulmonary TB, and case 2 involved a subject diagnosed with extrapulmonary TB. In case 1, the subject with initial indications was suspected of pulmonary TB. After undergoing ethambutol detection procedures and planar imaging at 1 hour and 4 hours post-radiopharmaceutical injection, the results showed pathological radioactivity uptake in the right apical lung mass and consolidation around the right medial lobe cavity on the 1-hour image, which increased on the 3-hour image. Case 1 subject was diagnosed with M.TB infection in the lungs. The SPECT/CT image showed an orange arrow indicating the location of the lung organ infected with M.TB. In case 2, the subject with initial indications was suspected of extrapulmonary TB in the glands. After undergoing ethambutol detection procedures and planar imaging at 1 hour and 4 hours post-radiopharmaceutical injection, the results showed pathological radioactivity uptake that persisted over time in the projection of the right neck lymph node glands. Case 2 subject was diagnosed with TB lymphadenitis. The static scan image of the neck region showed a green arrow indicating the location of the glands infected with M.TB. Previous studies have indicated that the ^{99m}Tc-ethambutol radiopharmaceutical can be used to detect M.TB in pulmonary TB cases [21]. In another study, ^{99m}Tc-ethambutol scintigraphy was used to diagnose extrapulmonary TB disease [17]. Imaging studies of ^{99m}Tc-ethambutol in experimental animals have shown that radioactivity uptake in the lungs is a positive sign of M.TB infection in the lungs [13]. Clinical evaluation of the use of ^{99m}Tc-ethambutol for TB disease diagnosis stated that gamma camera imaging shows the accumulation of radiotracer in lung organs, indicating infection in lung parenchyma due to M.TB.[10] Based on previous research, the use of ethambutol medication for TB disease therapy is the most effective effort in TB treatment [22]. It can be stated the effectiveness of ^{99m}Tc-ethambutol for diagnosing patients with TB with high accuracy within 1 hour post-radiopharmaceutical injection. Based on these two cases presented, it can be stated that the clinical evaluation of ^{99m}Tc-ethambutol for diagnosing pulmonary TB and extrapulmonary TB shows good results.

Based on the summary table of TB examination methods with treatment, there were 6 subjects with positive pulmonary TB results and 3 subjects with positive extrapulmonary TB results. There were 13 subjects with negative TB results. When ethambutol detection in subjects yielded positive results, it was reinforced by positive X-ray examination methods and positive culture results. However, there were 3 subjects where

ethambutol detection yielded positive results, and X-rays also yielded positive results, but culture examinations yielded negative results. These cases were found in subjects with extrapulmonary TB conditions, namely: 1 subject with TB in the right ilium bone, 1 subject with TB in the C5 Th1 vertebral area, and 1 subject with TB lymphadenopathy. Culture testing did not yield positive results because the examination was performed by culturing bacteria from the subject's sputum. In extrapulmonary TB conditions, the subjects' sputum did not contain M.TB, so when cultured, M.TB did not grow on the culture medium, but was detected by the ethambutol detection method. This is in line with previous research, where imaging yields positive results in lesions infected with M.TB, indicated by radioactivity uptake in lung or extrapulmonary organs/tissues.[6] In another study, the imaging yields TB negative results when there is no persistent radioactivity uptake in lung lesions (in lung region projections) or other organs [23].

Test results of *chi-square* and *phi coefficient* of ethambutol detection showed a relationship between ethambutol detection methods and post-treatment actions. The correlation value between them is 1.00, indicating a perfect correlation. Test results of *chi-square* and *phi coefficient* of thoracic/lumbosacral X-rays showed a relationship between thoracic/lumbosacral X-ray testing methods and treatment. The correlation value between them is 1.00, indicating a perfect correlation. Test results of *chi-square* and *phi coefficient* of culture testing showed a relationship between culture testing methods and post-treatment actions. This is supported by the correlation value between them, which is 0.736, indicating a high correlation. These statistical testing principles are in line with previous statistical research [15]. In chemical structure modeling, the binding of ^{99m}Tc-ethambutol to M.TB receptors in the body and its interaction and binding abilities constitute the detection capability of ^{99m}Tc-ethambutol [5]. It can be stated that ^{99m}Tc-ethambutol is a complex ligand chemical structure that has the ability to interact and bind with M.TB in the body, and with the help of gamma cameras, the location of radioactivity uptake can be determined as the location of M.TB-induced infection [7].

Ethambutol test scintigraphy, with a sensitivity of 100%, specificity of 100%, and accuracy of 100%. The higher the sensitivity value of the examination method, the better the examination method is in detecting actual patient cases, thus reducing the risk of false negative results. The higher the specificity value of the examination method, the better the examination method is in identifying true negative patients, thus reducing the risk of false positive results. Therefore, specificity measures how well the examination method can accurately differentiate healthy cases. The higher the accuracy value of the examination method, the better the examination method is in correctly distinguishing between the two types of cases, in this case positive or negative TB, thus reducing the risk of incorrect results. This is in line with the level of ethambutol detection scintigraphy reaching over 90% in spinal TB disease management [16]. It can be stated that TB examination with ethambutol test results in better diagnosis compared to other methods [6]. Based on previous research, ethambutol detection scintigraphy values are very high in diagnosing cases of TB arthritis and extrapulmonary TB [17]. Ethambutol test scintigraphy values are very high in spinal TB case management [16]. ^{99m}Tc-MIBI scintigraphy values are very high in managing pulmonary TB cases [23]. It can be stated that ethambutol test has a very high scintigraphy value, can be used for the diagnosis of intra and extrapulmonary TB, and can simultaneously detect.

The compliance test results for medical enforcement are 100%. This calculation is based on ethambutol detection results with positive treated outcomes or negative untreated outcomes. Ethambutol detection in molecular nuclear medicine and radiology provides diagnostic accuracy in difficult cases [24]. ^{99m}Tc-ethambutol is very useful in imaging TB lesions and is highly accurate [8]. ^{99m}Tc radiopharmaceutical chemistry is very good for perfusion imaging of the heart with compound complexes with MIBI [25]. It can be stated that ethambutol test accuracy is very high, and in some cases exceeds the initial suspicions of doctors during history taking, especially in cases of extrapulmonary TB. In this study, ethambutol test is very specific in detecting M.TB in infected lesions located throughout the body's organs/tissues.

What's particularly interesting about this study is that out of 22 subjects studied, 16 subjects had cancer conditions. Some findings obtained in the study include: ^{99m}Tc-ethambutol radiopharmaceuticals can be safely administered to immunocompromised patients. TB examination using ^{99m}Tc-ethambutol radiopharmaceuticals in immunocompromised patients did not reveal any drug side effects or complaints in the studied subjects. ^{99m}Tc-ethambutol as a radiotracer is not influenced by pre-existing conditions of patients suffering from cancer or tumors. This is consistent with research on radiopharmaceutical therapy, which has proven to be effective cancer treatment with minimal toxicity [26].

In addition to having very high scintigraphy, the ethambutol method has many advantages compared to other TB examination methods, namely: rapid examination within 1 hour, subjects can determine whether

they have TB or not; does not require invasive procedures for patients suspected of extrapulmonary TB, compared to TB examination by culture method, which requires suspected fluid specimens infected with M.TB from patients undergoing surgery [6]. Ethambutol test provides many benefits for patients diagnosed with extrapulmonary TB, patients do not need to undergo examination of organs/tissues suspected of being infected with M.TB or undergo invasive surgical procedures, patients only need to be given a radiotracer and undergo rapid imaging [27].

Complete blood examination results from all subjects showed normal results, both before and after injection of ^{99m}Tc-ethambutol radiopharmaceuticals. The complete blood parameters measured were hematology (hemoglobin, hematocrit, erythrocytes, platelets, leukocytes, MCV, MCH, and MCHC), SGOT, SGPT, urea, creatinine, peripheral blood morphology, before and after injection of ^{99m}Tc-ethambutol radiopharmaceuticals, no abnormal data were found from the blood reference value standards. Reference values: hematology: hemoglobin 12.5-17.3 g/dL (male) and 11.8-15.4 g/dL (female); hematocrit 38.1-50.4% (male) and 31.4-49.7% (female); erythrocytes 4.2-6.2 million/µL; platelets 150,000-500,000/µL; leukocytes 4,400-10,000/µL; MCV 80.1-94.3 fL, MCH 25.9-31.9 pg; MCHC 31.4-35.2 g/dL; SGOT: 5-40 U/L and SGPT 7-56 U/L; urea: 14-39 mg/dL (male) and 12-33 mg/dL (female); creatinine: 0.64-1.36 mg/dL; peripheral blood morphology: basophil 0-1; eosinophil 1-3; band neutrophil 2-6; segmented neutrophil 50-70; lymphocyte 20-40; monocyte 2-8 [6]. Monitoring of drug side effects based on complete blood examination results did not find any drug side effects before and after subjects were injected with ^{99m}Tc-ethambutol. In biological testing of ^{99m}Tc-ethambutol radiopharmaceuticals in animal models, no changes in blood characteristics were found over a period of 14 days [13]. Other research states that the physicochemical characteristics of ^{99m}Tc-human serum albumin (HAS)-nanospheres radiopharmaceuticals have no effect on liver organs, blood osmotic pressure, blood plasma concentration, or blood volume.[28] Based on previous research, minimal or no radiotracer side effects occur because the radiotracer dose given to subjects is only 2 mg and is excreted through physiological processes within 24 hours [16]. Research on the effects of administering anti-tuberculosis drugs to TB patients on blood to infected lesions found no other effects besides the therapeutic effects of the drug [30]. It can be stated that in this study, the ^{99m}Tc-ethambutol radiopharmaceutical did not alter blood characteristics before and after injection in all subjects, no medicine side effects were found.

Monitoring of complaints from all subjects showed normal results without complaints, both before and after injection of ^{99m}Tc-ethambutol radiopharmaceuticals. The monitored complaint parameters were upper abdominal pain, nausea, vomiting, dizziness, joint pain, headaches, fatigue, sore throat/mouth dryness, and red/itchy skin.[6] No subjects had complaints before or after injection of ^{99m}Tc-ethambutol radiopharmaceuticals. In previous research, ^{99m}Tc-ethambutol radiopharmaceuticals did not cause toxic effects [5]. Safety research on ^{99m}Tc-ethambutol radiopharmaceuticals in pediatric subjects found no side effects or complaints. In previous clinical evaluations, it was stated that no side effects emerged from one hundred and sixty-eight subjects involved in the study of the diagnostic value of ^{99m}Tc-ethambutol scintigraphy in TB compared to microbiological and histopathological tests [6]. Based on previous radiopharmaceuticals research marked by ^{99m}Tc, preparations did not cause side effects post-injection [29]. In the development of ^{99m}Tc-ethambutol-labeled compounds for TB diagnosis, no changes in physicochemical and microbiological characteristics were found in subjects [11]. It can be stated that the administration of ^{99m}Tc-ethambutol radiopharmaceuticals at 1 hour, 4 hours, up to 24 hours post-injection, did not find any subjects showing complaints.

5. CONCLUSION

The conclusion of this clinical evaluation research is that ^{99m}Tc-ethambutol can be used to diagnose both pulmonary and extrapulmonary TB. The scintigraphy value shows sensitivity of 100%, specificity of 100%, and accuracy of 100%. No side effects of the drug and subject complaints were found before and after the injection of ^{99m}Tc-ethambutol.

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