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Original Article

The Correlation between Plasma IL-17 Levels, Lymphocyte Counts, and Neutrophil Counts with the Colonisation of Candida sp. in Tuberculosis Patients with a Treatment History

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ABSTRACT

Introduction: *Tuberculosis (TB) patients with a history of ATT therapy are associated with changes in IL-17 levels, lymphocyte counts, and neutrophil counts. Increased IL-17 levels, lymphocyte counts and neutrophil counts indicate colonisation of Candida sp. fungus. Changes in the immune response in TB patients with Candida sp. colonisation may cause complications of TB that affect the treatment success rate.* ***Methods:*** *This research was designed for observational analytical study with a cross-sectional design. 59 subjects are divided into 3 groups, consisting of 21 TB-positive people with Candida sp. colonisation, 21 TB-positive people without Candida sp. colonisation, and 17 TB-negative people with Candida sp. colonisation. Plasma IL-17 levels are examined using the ELISA test, while the lymphocyte and neutrophil counts are seen from previous study examinations (AFFECT).* ***Results:*** *The plasma IL-17 levels in the TB-positive group with Candida sp. colonisation were 24.05 pg/ml (IQR 21.77-30.50). The plasma IL-17 levels in the TB-positive group without Candida sp colonisation were 23.08 pg/ml (IQR 19.11-32.46). The plasma IL-17 levels in the TB-negative group with Candida sp. colonisation is 20.72 pg/ml (IQR 18.51-22.84) pg/ml, (p=0.046). However, there*

are no statistically significant difference was observed in lymphocyte and neutrophil counts (p=0.078). ***Conclusions:*** *The differences in IL-17 levels that occur in the TB group with Candida sp colonisation may serve as an immunological signal suggesting the need for fungal assessment if the TB patients do not improve their treatment outcome after undergoing ATT for over 6 months.*

1. INTRODUCTION

Tuberculosis (TB) is an infectious disease that remains a global health issue. In addition to the increasing number of cases, the TB treatment failure adds more weight to the problems encountered.[1,2] Administering *Antitubercular therapy* (ATT), as a measure to treat TB, is known to cause an imbalance in normal flora, such as *Candida albicans*.[3,4]

Based on the Priority Fungal Pathogen list by *World Health Organization* (WHO), *Candida* sp. belongs to the critical group and high group – groups of fungi that frequently cause opportunistic infections that lead to death.[3–5] In this regard, prevalence data obtained from Soetomo Hospital in Surabaya suggest that over 50% (54.05%) of *Candida* sp. is identified in the sputum of TB patients.[6] another research shows that a group of TB patients undergoing prolonged ATT treatment has a high prevalence of *Candida* sp. colonisation (95%).[7]

Another issue that may arise in the course of TB is fungal infection. This is related to changes in immune conditions that occur in TB patients. The risk of haematological problems, such as decreasing Hb level (anaemia), and increasing or decreasing number of cells, such as lymphocytes and neutrophils may occur in TB patients.[8] Such a decrease in immune response affects the number of lymphocyte cells that produce pro-inflammatory cytokines, such as IL-17, which is known to be one of the immune responses involved in defence against Candida sp, and causes an imbalance in the body's normal flora due to the use of ATT that can be a predisposing factor for the increasing growth of *Candida* sp.[9–11]

Aspergillus and *Candida* are the types of fungi frequently found in the sputum of TB patients.[7,12] *Aspergillus* sp. may cause aspergilloma that bears clinical signs and symptoms similar to those of TB. Therefore, it is often misdiagnosed as recurrent TB. In the AFFECT research conducted by Dewi, et al – the protection for this research, concentration and clinical correlation of anti-Aspergillus IgG is examined in a group of chronic pulmonary TB patients at RSHS Bandung.[13] From the results of the sputum examination in this research, not only is a positive culture found for *Aspergillus* sp. but also *Candida* sp.

The risk of changes in immune response components, such as lymphocytes, neutrophils, and IL-17 caused by prolonged ATT therapy as a treatment for TB patients, becomes a predisposing factor for increased colonisation of *Candida* sp.[9,14–17] To date, no study has specifically evaluated differences in IL-17 levels and their associations with lymphocyte and neutrophil counts in TB patients with and without *Candida sp.* colonization following ATT. Therefore, in this research, a further study is conducted

regarding the *Candida sp.* colonisation found in the previous research. The idea is to identify the difference in IL-17 levels in groups with and without *Candida sp*. colonisation, as well as how this relates to the lymphocytes and neutrophil counts in TB patients with a treatment history. *Candida sp*. colonization may adversely affect the clinical prognosis of patients with tuberculosis (TB), affect treatment adherence, and increase the risk of secondary infections—yet the underlying immunological mechanisms remain poorly understood in treated TB patient populations.

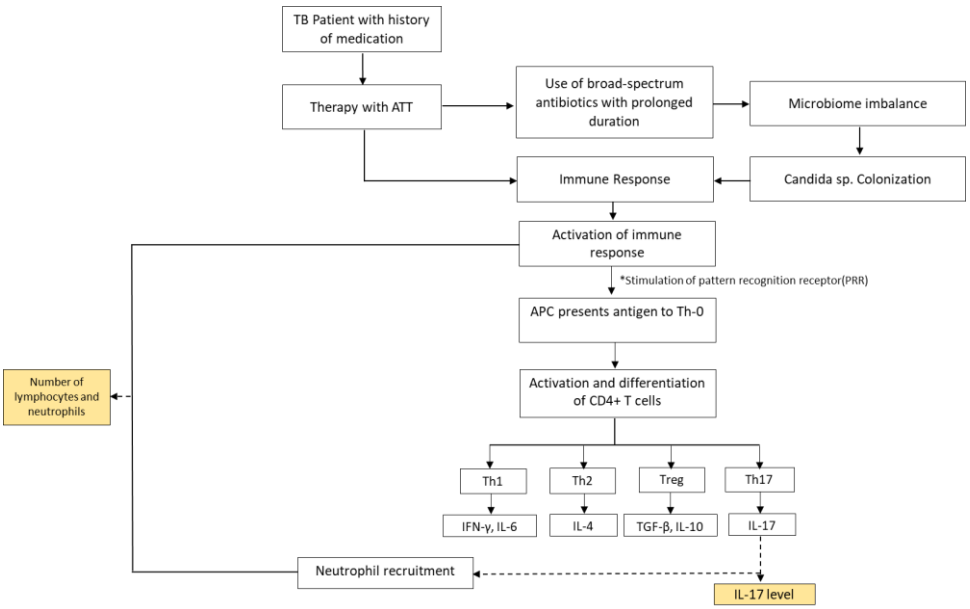

Figure 1. Conceptual Framework of the IL-17–Lymphocyte–Candida Interaction Pathway in Tuberculosis Patients with ATT history.

2. METHODS

This research is a non-experimental (analytic observational) type with a cross-sectional design to analyse the correlation and differences in IL-17 levels, lymphocyte cell counts, and neutrophil cell counts in TB patients with a treatment history who experience Candida sp colonisation and those who do not experience *Candida* sp. colonisation. The population of this research are TB patients included in the research history entitled AFFECT (*Aspergillus fumigatus* in chronic TB).[13]

This research analysed three immunological parameters, namely IL-17 levels, lymphocyte counts and neutrophil counts. IL-17 levels examination is performed using the *Enzyme-Linked Immunosorbent Assay* (ELISA) method. The samples used in this research were archieved biological material in the form of plasma from the aforementioned research.[13] Absolute lymphocyte and neutrophil counts were quantified using a hematology analyser, which may not fully assess functional or subpopulation-specific immune responses in TB–Candida colonisation.

Samples collection for the AFFECT study was conducted in 2018. The samples were stored at -80°C and had never undergone repeated freeze-thawing to preserve sample quality.

The archieved plasma used in this research must comply with the criteria for an ELISA examination and so must the secondary data that include patient demography, TB treatment history (type of ATT, duration of treatment, number of previous TB

episodes, and medication status), as well as the supporting data, such as microbiology test results (TB diagnosis), fungal culture results, *Body Mass Index* (BMI), lymphocyte counts, dan neutrophil counts to be used in the analysis. In this research, *Candida* identification was based solely on fungal culture results, with isolates differentiated on CHROMagar medium. Colonisation by *Candida* was defined by positive culture growth, establishing true infection requires clinical evidence and additional laboratory investigations, which were not performed in the present study.

The sample size was calculated using a group comparison formula, resulting in a total of 21 subjects per group. The sample was divided into three groups, namely the TB-positive group with *Candida* colonisation, the TB-positive group without *Candida* colonisation, and the TB-negative group with *Candida* colonisation after obtaining secondary data that meet the criteria. However, during sample screening, we did not obtain the required number of samples in the TB-negative with Candida group, as only 17 samples had positive results in the Candida fungal culture. This imbalance in sample sizes may impact the statistical results of the study.

Once the samples have been prepared, IL-17 is examined using ELISA (ELISA Kit for human IL-17 (Wuhan, Fine Biotech Co., Ltd. No. Kat.EH4461) with intra- and inter-assay variation coefficients are 5.87% and 5.57%. Then, data analysis is performed on the obtained IL-17 levels, lymphocyte counts, and neutrophil counts.

Statistical analysis of research data begins with a data normality test using Kolmogorov Smirnov. Data on the characteristics of archieved biological material owner subjects are analysed using a frequency distribution with units of proportion/percentage. The results of immunological data analysis on IL-17 levels, lymphocyte counts, and neutrophil counts are presented in median and quartile 1(Q1) – quartile 3 (Q3). Data analysis is performed using Kruskal-Wallis and continued with Post Hoc analysis. To identify the correlation between IL-17 levels and the lymphocyte and neutrophil counts, analysis with Spearman's rank correlation is performed. The test result significance is determined by the value of $p<0,05$ and data processing is performed using the IBM SPSS program version 27.0

3. RESULTS

This research was conducted between October 2023 and June 2024, beginning by collecting archieved biological material data from the plasma of patients included in the AFFECT study.[13] The plasma of patients who meet the inclusion criteria is sent to the Immunology Laboratory, Faculty of Medicine, Padjadjaran University for an IL-17 level test using the ELISA method. The samples in this research are divided into 3 groups, namely the TB group with *Candida* colonisation, TB without *Candida* colonisation, and TB negative with Candida colonisation. The results of the sample size calculation for this research are 21 for each group. However, when collecting samples in the TB negative group with *Candida*, only 17 samples meet the inclusion criteria. Therefore, the total number of samples in this research is 59.

The results of research subject characteristics show that the highest mean age is 48.18$\pm$12.28 years old in the TB-negative group with *Candida* colonisation, followed by the TB-positive group with *Candida* colonisation which has a mean age of 43.86$\pm$11.62 years old. The lowest mean age is in the TB-positive group without *Candida* colonisation, namely 35.43$\pm$11.63 years old.

Table 1. Data of the characteristics of the research subjects

Characteristics	TB (+)		TB (-)
	With *Candida sp* colonisation (n=21)	Without *Candida sp* colonisation (n=21)	With *Candida sp* colonisation (n=17)
	n (%)	n (%)	n (%)
Age (year)			
15-24	2 (9.5%)	5 (23.8%)	1 (5.9%)
25-34	2 (9.5%)	5 (23.8%)	3 (17.7%)
35-44	7 (33.3%)	6 (28.6%)	2 (11.8%)
45-54	7 (33.3%)	3 (14.3%)	5 (29.4%)
55-64	2 (9.5%)	2 (9.5%)	6 (35.3%)
≥ 65	1 (4.8%)	0 (0%)	0 (0%)
Age (year, mean ± SD)	43.86 ±11.62	35.43±11.63	48.18±12.28
Gender			
Male	12 (57.1%)	13 (61.9%)	11 (64.7%)
Female	9 (42.9%)	8 (38,1%)	6 (35.3%)
Body Mass Index [(kg/m^2, median (Q1,Q3)]	18.73 (12.07-25.65)	18.59 (14.79-28.0)	20.02 (13.79-27.94)
***Candida* Species**			
Candida albicans	17 (81%)	0 (0%)	14 (82.4%)
Candida non-albicans	3 (14.3%)	0 (0%)	2 (11.8%)
Mixed Candida	1 (4.8%)	0 (0%)	1 (5.9%)
Types of TB Treatment			
ATT category 1	13 (61.9%)	17 (81%)	12 (57.1%)
ATT category 1 and 2	8 (38,1%)	4 (19%)	5 (23.8%)
Duration of TB Treatment (months)			
< 6	0 (0%)	1 (4.8%)	0 (0%)
6	3 (14.3%)	8 (38.1%)	6 (35.3%)
> 6	18 (85.7%)	12 (57.1%)	11 (64.7%)
Previous TB Episode			
1	9 (42.9 %)	15 (71.4%)	11 (64.7%)
>1	12 (57.1%)	6 (28.6%)	6 (35.3%)
Taking ATT during Examination			
Yes	3 (14.3%)	4 (19%)	5 (23.8%)
No	18 (85.7%)	17 (81%)	12 (57.1%)
TB Diagnosis Method			
Smear microscopy			
Positive	13 (61.9%)	12 (57.1%)	0 (0%)
Negative	8 (38.1%)	9 (42.9%)	17 (100%)
GeneXpert MTB-RIF			
Rif-sensitive	4 (19%)	2 (9.5%)	0 (0%)
Rif-resistant	17 (81%)	19 (90.5%)	0 (0%)
Negative	0 (0%)	0 (0%)	17 (100%)
Mtb culture			
Positive	18 (85.7%)	17 (81%)	0 (0%)
Negative	3 (14.3%)	4 (19%)	17 (100%)

Of the *Candida* species identified from the sputum examination results in this research, the majority are found to be albicans, then non-albicans species. Over 80% of sputum is confirmed Candida albicans species in both groups with *Candida* colonisation. Meanwhile, the non-albicans species found include *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis*.

Concerning the previous TB history, the majority of research subjects have a TB treatment history with a duration of over 6 months (41/59) and are dominated by the TB-

positive group with *Candida* colonisation, namely 18 people (85.7%). Subjects in this research also have a history of experiencing more than one previous TB episode. This happens to 24/59 research subjects from the three groups and over 50% come from the TB-positive group with *Candida* colonisation. Most of the research subjects are known to be out of the medication process at the time of examination in the previous study.

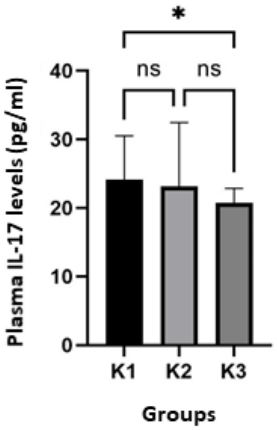

Figure 2. IL-17 level difference test between groups

Based on Table 1, the groups with the highest significance values occur in the TB-negative group with *Candida* colonisation and the TB-positive group with *Candida* colonisation. According to the graph, there are 4 subjects with IL-17 levels above the median for each group [24.05 pg/ml (IQR 21.77-30.50), 23.08 pg/ml (IQR 19.11-32.46), and 21.72 pg/ml (IQR 18.51-22.84)].

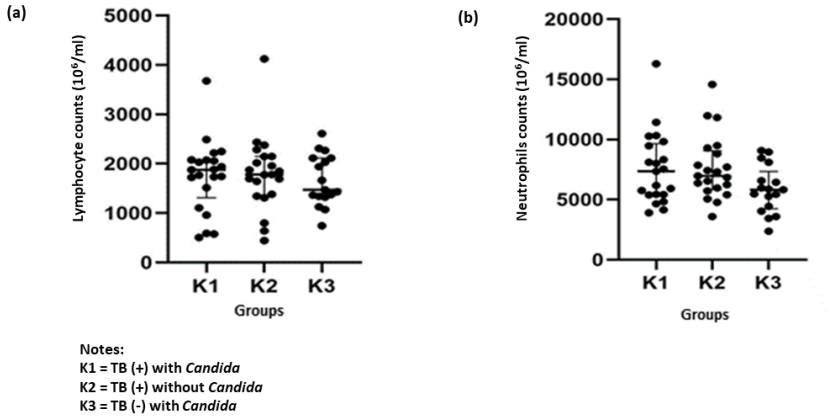

Figure 3. Lymphocyte and neutrophil count difference test between groups

The Kruskall-Wallis difference test results suggest that there are no significant differences in either lymphocyte counts or neutrophil counts from the three research groups. Figure 2 shows that several subjects have lymphocyte and neutrophil counts above the median. As seen, two have lymphocyte counts above the median of the TB-positive group with Candida (1877.5x10^6/ml) with lymphocyte counts of $\pm$3600x10^6/ml and above the median of the TB group without Candida (1782x10^6/ml) with lymphocyte counts of $\pm$4100 x10^6/ml.

Table 2 Correlation between IL-17 levels, lymphocyte counts, and neutrophil counts

Variable	Correlation Coefficient (r)	Value-p
IL-17 and lymphocyte counts	0.009	0.948
IL-17 and neutrophil counts	0.063	0.634
lymphocyte counts and neutrophil counts	0.238	0.072

The correlation between IL-17 levels, lymphocyte counts and neutrophil counts is seen from the correlation test using Spearman's rank. Table 2 suggests that there is no correlation between IL-17 and lymphocyte counts (0.948, 0.009), IL-17 and neutrophil counts (0.634, 0.063).

4. DISCUSSIONS

4.1 TB–Candida Coinfection

Candida colonisation in TB patients has been widely observed and *Candida albicans* is the most commonly found species.[18,19] A weakened immune system in TB patients creates opportunities for *Candida* to codevelop with Mycobacterium tuberculosis, thereby exacerbating the patient's condition.[20]

Several studies state that *Candida* colonisation in TB patients is related to the duration of TB treatment. A study conducted on TB patients with a 6-month ATT treatment identifies abnormal colonisation of *Candida* sp, compared with patients who are resistant to ATT and require prolonged antibiotic treatment (8 to 20 months).(12) Similar to this research, it is found that over 80% of TB patients with Candida colonisation have a history of more than 6 months of ATT treatment and experience more than 1 TB episode. Similarly, research in North Sulawesi suggests that there is a significant correlation between the duration of TB treatment and *Candida* colonisation (p=0.028). This is also supported by Astekar et al and Soedarsono et al who state that TB patients undergoing prolonged ATT therapy have a lower prevalence of *Candida* coinfection than those without ATT treatment.[6,7]

In terms of age, a difference test is performed on the age among the three groups and there is a significant difference ($p<0.05$). When the ages of the three groups are compared, the mean in the TB group without *Candida* colonisation tends to be younger (35.43 ± 11.63 years old) than the group with *Candida* colonisation (48.18 ± 12.28 years old). This indicates that age can be a risk factor for TB and *Candida*. Research conducted by Utami et al suggests that age is a TB risk factor.[21] In their research, the TB prevalence is found higher in subjects under 45 years old (72.6%). This group is of productive age and tends to transmit TB due to high mobility thereby the possibility of being exposed to *Mtb* is greater while at older ages, endogenous reactivity may occur due to declining physical condition thus the body's immune system cannot counter *Mtb* bacteria. On the other hand, research conducted by Amiri M. et al suggests that Candida colonisation with TB occurs in older patients (>40 years old). In their research, the highest *Candida* colonisation frequency in TB patients is reported in the 41-50 years age group. Therefore, with older ages, TB frequency with fungal colonisation increases and is statistically significant.[18]

The TB development and prolonged antibiotic treatment make TB patients susceptible to the development of fungal colonisation into infection.[19,22] A study in Asia

shows that *Candida* coinfection is prevalent in TB patients and *Candida albicans* is the most commonly identified species with a percentage exceeding 50%.[19] Common coinfections that occur globally in TB patients are coinfections with Candida and HIV.[22,23] The patient prevalence with TB/HIV in some countries is approximately 80%,[23] while TB patients coinfected with *Candida sp* were about 15-32%.[22]

The alteration from *Candida* colonisation into infection may be affected by prolonged antibiotic treatment and is exacerbated when an immunodeficiency condition occurs in TB patients.[19] Antibiotics as TB therapy may cause environmental changes and microbiome imbalance. Prolonged ATT administration to TB patients suppresses normal flora and allows the growth of opportunistic fungi.[24] This is related to the decline in microbial ecosystems, such as *Lactobacillus* or *Bifidobacterium*. Research conducted by Cao et al states that ATT therapy can reduce the number of bacteria beyond which is known to maintain fungal growth.[25] This microbiome imbalance causes opportunistic fungi to turn into pathogens.[24]

In addition to the aforementioned factors, local factors, such as poor oral hygiene, use of removable dentures, xerostomia, smoking habits, and a high carbohydrate diet, may also facilitate the occurrence of candidiasis.[4] Based on these matters, the correlation between TB and susceptibility to abnormal *Candida* colonisation is related to immunological and environmental factors that support *Candida* coinfection.[3]

4.2 Immunological Mechanism (IL-17 axis)

Various factors may affect IL-17 levels, lymphocyte counts and neutrophil counts. In this research, statistical tests are performed on various research subjects' characteristics that may cause differences in IL-17 levels, lymphocyte counts, and neutrophil counts in the research group, such as age, duration of treatment, and ATT medication status.

The correlation between age, duration of treatment, and ATT medication status with IL-17 levels is analysed in this research to avoid bias factors that may affect the results due to differences in IL-17 levels of the research groups. Based on the statistical test results to identify the correlation between age and duration of treatment performed in the three research groups, no statistically significant difference was observed ($p>0.05$). Therefore, this research suggests that differences in IL-17 levels that occur in the two groups are not biased by age and duration of treatment.

Lymphocytes are known to have an important role in the human immune system which function as an immunoregulator and immunomodulator in the immune system. Lymphocytes are categorised into T cells and B cells.[26] IL-17 is known to be an immune response that has a role against *Candida* sp. which is produced by T cell lymphocytes. T cells are activated and differentiated into Th-17 and produce IL-17. When *Candida* colonisation builds up, such as on the oral mucosa, the IL-17 production increases and controls the neutrophil mediation of the inflammatory response, intensifying the barrier function of the epithelial tissue in the mucosa as a host defence against *Candida*.[15,16,27] However, when IL-17 activity in the host, this may escalate the risk of infection by *Candida sp.*[28]

In addition to producing IL-17, Th-17 also produces IL-22 which has a role against Candida.[29] Both cytokines mediate antifungal immunity by activating rather different signalling pathways in epithelial tissue. IL-22 signaling through STAT-3 induces BEL cell

proliferation. The signal depending on IL-22 is required for SEL renewal which is responsive to IL-17. Therefore, IL-22/STAT3 signalling indicates a response to IL-17 signalling in the oral epithelium despite acting on different epithelial layers.[29]

IL-17 stimulates neutrophil requirement and encourages AMP production which has a role in the immune response against *Candida* sp. Neutrophils limit C. albicans growth through ROS after recognising *C. albicans* through Dectin-1. Neutrophil cells can also form NET in response to *C. albicans*. NET is a fibril network structure containing DNA and various granular proteins released by neutrophils as a defence response to fungi.[30] Based on this matter, a correlation test is conducted in this research to identify whether there is a correlation between IL-17 and the number of lymphocytes that produce IL-17 as well as the number of neutrophils which can be produced by IL-17. Furthermore, an analysis of different immunological parameter tests is performed in the three research groups.[15] However, based on the statistical analysis performed in this research, no differences or correlations between IL-17, lymphocyte counts, and neutrophil counts are found in patients with a history of TB treatment, with or without Candida colonisation.

Examination of IL-17 levels in this research is also conducted to see differences in IL-17 levels in TB patients who experience *Candida* colonisation and those who do not. IL-17 levels increase due to colonisation [24.05 pg/ml (IQR 21.77-30.50), 23.08 pg/ml (IQR 19.11-32.46), and 21.72 pg/ml (IQR 18.51-22.84)]. Post-hoc analysis further indicated that IL-17 levels were significantly higher in the TB-positive group than in the TB-negative group among patients with previous TB treatment and Candida colonisation (p=0.014).Research conducted by Chimens et al even shows that in subjects with *Candida* fungal infections, the plasma level of IL-17 doubles that of healthy subjects.[27] The results of this research suggest that there are IL-17 level differences in patients with a TB treatment, between the TB-positive group and the TB-negative group with *Candida* sp. colonisation (p=0.046). However, no statistically significant difference was observed in lymphocyte and neutrophil counts (p=0.078).

4.3 Methodological Limitations

This research is limited by a small and unbalanced sample size, which significantly compromises the validity and reliability of the findings. Parameter estimates, such as means and proportions, exhibit instability due to high sampling variability, yielding wide confidence intervals and consequently reduced statistical power. Furthermore, the imbalance in sample composition in the TB(+) Candida(-) group—comprising only 17 of 21 samples in group—may introduce selection bias and uncontrolled confounding variables, potentially violating the assumptions of parametric tests and confounding the interpretation of associations between variables.

The lack of correlation and statistically significant differences in lymphocyte and neutrophil counts may further be attributed to the limited specificity of the immunological assessment methods employed. In this research, absolute lymphocyte and neutrophil counts were determined using a haematology analyser, which may not adequately capture functional or subpopulation-specific immune responses relevant to TB and Candida co-infection. In this research, the absolute number of lymphocytes and neutrophils is calculated using a haematology analyser. Regarding the analysis results on lymphocyte counts in this research, it happens because the absolute number of

lymphocytes measured is the result of its calculation in the bloodstream instead of the specific Th-17 lymphocyte that produces IL-17. Therefore, it cannot show a linear correlation between increasing levels of IL-17 with measurable lymphocyte counts.[31] Similarly, the number of neutrophils calculated using a haematology analyser is less capable of specifically detecting and assessing the function of neutrophils against *Candida*. In addition, the haematology analyser method that measures neutrophil counts may not be able to detect neutrophils activated by IL-17 and *Candida*. Therefore, the measurable neutrophil counts are non-linear with increasing IL-17 levels.[32] Based on research conducted by Geissal et al, automatic haematology analyser often fails to detect immature neutrophils, such as not detecting 24% of neutrophil band forms.[32] Furthermore, research conducted by Gioia et al states that neutrophil examination results using a haematology analyser – despite showing an increase in neutrophil counts due to *Candida*, cannot be specified for infection by *Candida*.[33] Therefore, other methods are required to identify *Candida*, such as microscopic confirmation as in the immunofluorescence method (IF). This method is capable of identifying antigen-antibody complex bonds under an ultraviolet microscope using specific antibodies labelled on fluorochromes.[33]

Several studies suggest that the use of another examination method, such as immunofluorescence, is known to be able to identify neutrophil activities through the NET formation in response to *Candida* by neutrophil.[34,35] Research conducted by Ayna et al states that flow cytometry is an effective method to evaluate the percentage of T cell and B cell lymphocytes.[36] The flow cytometry method is known to detect lymphocyte subsets that may help in clinical evaluation.[37] Research conducted by Le et al also suggests that this method is capable of counting lymphocytes and showing results with over 90% accuracy.[38]

The limitation of this research is that it is not conducted prospectively and longitudinally. Therefore, the research cannot identify the clinical outcomes of treatment results in TB patients with an ATT treatment history and *Candida* colonisation, which correlate to higher IL-17 levels. This research also focuses on performing IL-17 levels examination, thereby it cannot explain the correlation of the higher IL-17 levels in the research groups. This relates to T cell subsets or other cytokines related to IL-17 that are not examined in this research.

5. CONCLUSION

The IL-17 levels in TB patients with a treatment history who do and do not experience *Candida sp* colonisation have significant differences. In addition to no correlations found between lymphocyte counts, neutrophil counts, and IL-17 levels, there are no statistically significant difference was observed in the number of lymphocytes and neutrophils. Nevertheless, it is known that IL-17 levels in the TB-positive group with *Candida* colonisation are higher than in the other groups. This indicates that TB conditions with *Candida* colonisation cause a different inflammatory immune response, particularly with the characteristics of patients experiencing more than 1 TB episode and are resistant to ATT (rifampicin), leading to an increase in the duration of TB treatment. Therefore, further longitudinal research is required to identify the clinical outcome development of treatment for patients with a TB treatment history and *Candida sp*

colonisation who have higher IL-17 levels. This research and various prevalence data related to *Candida* colonisation in TB patients show the importance of conducting fungal examination in TB patients if treatment outcomes do not improve after administering ATT for more than 6 months to prove whether *Candida sp.* colonisation affects the treatment outcomes.

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REFERENCES

1. Kementerian Kesehatan RI. https://yankes.kemkes.go.id/. 2022. p. 1–3 Kepatuhan Pengobatan pada Tuberculosis.

2. Kementerian Kesehatan RI. Pedoman Nasional Tata Laksana Tuberkulosis. Kemenkes RI. 2020;1–156.

3. Vila T, Sultan AS, Montelongo-Jauregui D, Jabra-Rizk MA. Oral candidiasis: A disease of opportunity. Journal of Fungi. 2020 Mar 1;6(1):1–28.

4. Talapko J, Juzbašić M, Matijević T, Pustijanac E, Bekić S, Kotris I, et al. Candida albicans-the virulence factors and clinical manifestations of infection. Journal of Fungi. 2021 Feb 1;7(2):1–19.

5. World Health Organization. WHO fungal priority pathogens list to guide research, development and public health action. 2022.

6. Soedarsono S, Prasetiyo Y, Mertaniasih N. Fungal isolates findings of sputum samples in new and previously treated cases of pulmonary tuberculosis in dr. soetomo hospital surabaya, Indonesia. International Journal of Mycobacteriology. 2020 Apr 1;9(2):190–4.

7. Astekar M, Bhatiya PS, Sowmya G V. Prevalence and characterization of opportunistic candidal infections among patients with pulmonary tuberculosis. Journal of Oral Maxillofacial Pathology. 2016 May 1;20(2):183–9.

8. Abay F, Yalew A, Shibabaw A, Enawgaw B. Hematological Abnormalities of Pulmonary Tuberculosis Patients with and without HIV at the University of Gondar Hospital, Northwest Ethiopia: A Comparative Cross-Sectional Study. Tuberculosis Research and Treatment. 2018 Dec 30;20(18):1–6.

9. Xu L, Cui G, Jia H, Zhu Y, Ding Y, Chen J, et al. Decreased IL-17 during treatment of sputum smear-positive pulmonary tuberculosis due to increased regulatory T cells and IL-10. Journal of Translational Medicine. 2016 Jun 16;14(1):1–11.

10. DiNardo AR, Rajapakshe K, Nishiguchi T, Grimm SL, Mtetwa G, Dlamini Q, et al. DNA hypermethylation during tuberculosis dampens host immune responsiveness. Journal of Clinical Investigation. 2020 Jun 1;130(6):3113–23.

11. Hong BY, Maulén NP, Adami AJ, Granados H, Balcells ME, Cervantes J. Microbiome changes during tuberculosis and antituberculous therapy. Clinical Microbiology Reviews. 2016 Oct 1;29(4):915–26.

12. Maloho RF, Putri Solikah M. Hubungan jamur Candida albicans dan Aspergillus fumigatus terhadap pasien tuberkulosis paru di balaI Laboratorium Kesehatan Daerah (BLKD) Provinsi Sulawesi Utara. Jurnal Analisis Laboratorium Medis. 2023 Dec 19;8(2):108–16.

13. Dewi I, Soeroto AY, Putriyani G, Hanifah W, Permata A, Annisa J, et al. Aspergillus fumigatus-specific antibodies in patients with chronic tuberculosis. International Journal Tuberculosis Lung Disease. 2020 Aug 1;24(8):853–6.

14. Chedid C, Kokhreidze E, Tukvadze N, Banu S, Uddin MKM, Biswas S, et al. Association of baseline white blood cell counts with tuberculosis treatment outcome: a prospective multicentered cohort study. International Journal of Infectious Disease. 2020 Nov 1;100:199–206.

15. Conti HR, Gaffen SL. IL-17–Mediated Immunity to the Opportunistic Fungal Pathogen Candida albicans . J Immunol. 2015 Aug 1;195(3):780–8.

16. Huppler AR, Conti HR, Hernández-Santos N, Darville T, Biswas PS, Gaffen SL. Role of Neutrophils in IL-17–Dependent Immunity to Mucosal Candidiasis. Journal of Immunology. 2014 Feb 15;192(4):1745–52.

17. Lopes JP, Lionakis MS. Pathogenesis and virulence of Candida albicans. Virulence. 2022;13(1):89–121.

18. Amiri MRJ, Siami R, Khaledi A. Tuberculosis Status and Coinfection of Pulmonary Fungal Infections in Patients Referred to Reference Laboratory of Health Centers Ghaemshahr City during 2007-2017. Ethiopian Journal of Health Sciences. 2018 Nov 1;28(6):683–90.

19. Hadadi- Fishani M, Shakerimoghaddam A, Khaledi A. Candida coinfection among patients with pulmonary tuberculosis in Asia and Africa; A systematic review and meta-analysis of cross-sectional studies. Microbial Pathogenesis. 2020 Feb 1;139:1–7.

20. Orlandini RK, Rocha ACSD, Silva GA, Watanabe E, Motta ACF, Silva-Lovato CH, et al. Increased diversity, fungal burden, and virulence of oral Candida spp. in patients undergoing anti-tuberculosis treatment. Microbial Pathogenesis. 2021 Dec 1;161:1–12.

21. Utami F, Salam A, Handoko W. Hubungan usia, jenis kelamin-dan tingkat kepositifan dengan konversi basil tahan asam pasien tuberkulosis di unit pengobatan penyakit paru-paru Pontianak. 2014.

22. Fontalvo DM, Borre G, Camargo DG, Jimenez NC. Tuberculosis and fungal co-infection present in a previously healthy patient. Colombia Medica. 2016;47(2):105–8.

23. World Health Organization. Geneva : WHO Library Cataloguing-in-Publcation

Data. 2022. p. 1–10 Global tuberculosis report 2022.
24. Baumgardner DJ. Oral Fungal Microbiota: To Thrush and Beyond. J Patient-Centered Res Rev. 2019 Oct 28;6(4):252–61.

25. Cao D, Liu W, Lyu N, Li B, Song W, Yang Y, et al. Gut Mycobiota Dysbiosis in Pulmonary Tuberculosis Patients Undergoing Anti-Tuberculosis Treatment. Microbiology Spectrum. 2021;9(3):1–13.

26. Alley TF, Moscatello K. Immunology and Microbiology. New York: Kaplan, Inc; 2016. p. 3–13.

27. Chimenz R, Tropeano A, Chirico V, Ceravolo G, Salpietro C, Cuppari C. IL-17 serum level in patients with chronic mucocutaneous candidiasis disease. Pediatric Allergy and Immunology. 2022 Jan 1;33(S27):77–9.

28. Whibley N, Tritto E, Traggiai E, Kolbinger F, Moulin P, Brees D, et al. Antibody blockade of IL-17 family cytokines in immunity to acute murine oral mucosal candidiasis. Journal of Leukocyte Biology. 2016 Jun 1;99(6):1153–64.

29. Aggor FEY, Break TJ, Trevejo-Nuñez G, Whibley N, Coleman BM, Bailey RD, et al. Oral epithelial IL-22/STAT3 signaling licenses IL-17-mediated immunity to oral mucosal candidiasis. Science Immunology. 2020 Jun 5;5(48):1–30.

30. Zhou Y, Cheng L, Lei YL, Ren B, Zhou X. The Interactions Between Candida albicans and Mucosal Immunity. Frontiers in Microbiology. 2021 Jun 21;12:1–12.

31. Marits P, Wikström AC, Popadic D, Winqvist O, Thunberg S. Evaluation of T and B lymphocyte function in clinical practice using a flow cytometry based proliferation assay. Clinical Immunology. 2014;153(2):332–42.

32. Geissal ED, Coffey T, Gilbert DN. Clinical Importance of the Failure to Detect Immature Neutrophils by an Automated Hematology Analyser Background: Hospital clinical laboratories increasingly use automated. Infectious Diseases Clinical Practice [Internet]. 2010;18(6). Available from: www.infectdis.com

33. La Gioia A, Devito A, Fiorini F, Bombara M, Isola P, Spinale B, et al. Cytographic changes on BC-6800 Haematological Analyser related to the presence of Candida albicans in peripheral blood. A new tool to suspect candidemia? Journal of Clinical Pathology. 2017 Jun 1;70(6):494–9.

34. He Y, Liu J, Chen Y, Yan L, Wu J. Neutrophil Extracellular Traps in Candida albicans Infection. Front Immunol. 2022 Jun 16;13:1–7.

35. Stoimenou M, Tzoros G, Skendros P, Chrysanthopoulou A. Methods for the Assessment of NET Formation: From Neutrophil Biology to Translational Research. International Journal of Molecular Sciences. 2022 Dec 1;23(24):1–16.

36. Kılıçaslan Ayna T, Akman B, Özkızılcık Koçyiğit A, Güleç D, Tugmen C, Soyöz M. Flow cytometric evaluation of T and B lymphocyte percentage in chronic kidney disease. Medical Research Journal. 2017 Sep 21;2(1):29–33.

37. Rovati B, Mariucci S, Poma R, Tinelli C, Delfanti S, Pedrazzoli P. An eight-colour flow cytometric method for the detection of reference values of lymphocyte

subsets in selected healthy donors. Clinical and Experimental Medicine. 2014;14(3):249–59.

38. Le Dang-Khoa Tan, Bui Avy An, Yu Zexi. An automated framework for counting lumpocytes from microscopic images. Vancouver: Institute of Electrical and Electronics Engineers; 2015. p. 421.

Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.