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Correlation between neutrophil-to-lymphocyte ratio and molecular test Ct category in patients with drug-resistant tuberculosis

Korelasi antara Rasio Neutrofil-Limfosit dan Kategori Ct Tes Molekuler pada Pasien Tuberkulosis Resisten Obat

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ABSTRACT

Background: *Drug-resistant tuberculosis remains a clinical and public health challenge. The neutrophil-to-lymphocyte ratio (NLR) reflects systemic inflammation, whereas the cycle threshold (Ct) category from Xpert Mycobacterium tuberculosis/rifampicin (MTB/RIF) Ultra reflects semiquantitative molecular bacillary load. Evidence linking both parameters in drug-resistant tuberculosis remains limited.*
Objective: *To analyze the correlation between blood NLR and molecular test Ct category in patients with drug-resistant tuberculosis.*
Methods: *This analytic observational study with a cross-sectional design was conducted at Dr. H. A. Rotinsulu Lung Hospital, Bandung, from January to November 2024. Twenty-nine adult patients with drug-resistant tuberculosis who had complete hematology and Xpert MTB/RIF Ultra results were selected using purposive sampling. NLR was calculated from differential leukocyte counts. Ct was grouped into high, medium, and low categories. Data were analyzed using descriptive statistics, Spearman correlation, and the Kruskal-Wallis test.*
Results: *Of 29 patients, 19(66%) were male, and 22(76%) of the whole sample had elevated NLR. Ct categories consisted of high in 15 patients, medium in 3 patients, and low in 11 patients. Mean NLR was 6.76 in the high Ct group, 3.50 in the medium group, and 5.49 in the low group. Spearman analysis showed a very weak and non-significant positive correlation between NLR and Ct category (r=0.101; p=0.300). The Kruskal-Wallis test also showed no significant NLR difference across Ct categories (H=1.667; p=0.435).*
Conclusion: *NLR was not significantly correlated with Ct category and did not differ significantly across Ct groups. NLR should be interpreted as an adjunct inflammatory marker, not as a substitute for molecular bacteriological assessment in drug-resistant tuberculosis.*

Keywords: *cycle threshold, drug-resistant tuberculosis, molecular rapid test, neutrophil-to-lymphocyte ratio, Xpert MTB/RIF Ultra*

ABSTRAK

Latar Belakang: Tuberkulosis resisten obat masih menjadi tantangan klinis dan kesehatan masyarakat. *Rasio neutrofil-limfosit* atau *neutrophil-to-lymphocyte ratio* (NLR) menggambarkan inflamasi sistemik, sedangkan kategori *cycle threshold* (Ct) *Xpert Mycobacterium tuberculosis/rifampicin* (MTB/RIF) Ultra menggambarkan beban basil molekuler secara semikuantitatif. Bukti mengenai hubungan kedua parameter tersebut pada tuberkulosis resisten obat masih terbatas.

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Tujuan: Menganalisis korelasi antara NLR darah dan kategori Ct tes molekuler pada pasien tuberkulosis resisten obat.
Metode: Penelitian observasi analitik dengan desain *cross sectional* dilakukan di RS Paru Dr. H. A. Rotinsulu Bandung pada Januari-November 2024. Sampel terdiri atas 29 pasien dewasa tuberkulosis resisten obat dengan data hematologi dan Xpert MTB/RIF Ultra lengkap yang dipilih secara *purposive sampling*. NLR dihitung dari hitung jenis leukosit. Ct dikelompokkan menjadi kategori *high, medium*, dan *low*. Data dianalisis secara deskriptif, dilanjutkan uji korelasi Spearman dan uji *Kruskal-Wallis*.
Hasil: Dari total 29 pasien, 19 pasien (66%) berjenis kelamin laki-laki dan 22 pasien (76%) dari seluruh sampel memiliki NLR meningkat. Kategori Ct terdiri atas *high* pada 15 pasien, *medium* pada 3 pasien, dan *low* pada 11 pasien. Rata-rata NLR pada kelompok Ct *high* adalah 6,76, *medium* 3,50, dan *low* 5,49. Uji Spearman menunjukkan korelasi positif sangat lemah dan tidak bermakna antara NLR dan kategori Ct (r=0,101; p=0,300). Uji *Kruskal-Wallis* juga tidak menunjukkan perbedaan NLR yang bermakna antar-kategori Ct (H=1,667; p=0,435).
Kesimpulan: NLR tidak berkorelasi bermakna dengan kategori Ct dan tidak berbeda bermakna antar-kelompok Ct. NLR lebih tepat digunakan sebagai penanda inflamasi tambahan, bukan sebagai pengganti pemeriksaan bakteriologis molekuler pada tuberkulosis resisten obat.

Kata kunci: cycle threshold, neutrophil-to-lymphocyte ratio, tes cepat molekuler, tuberkulosis resisten obat, Xpert MTB/RIF Ultra

INTRODUCTION

Tuberculosis (TB) remains a leading cause of death from infectious disease.[1] Drug-resistant TB increases the burden of TB control because diagnosis, treatment, and monitoring are more complex than those of drug-susceptible TB. This condition requires laboratory indicators that help clinicians assess infection status and the patient's inflammatory response more comprehensively.[2]

Molecular rapid testing using Xpert MTB/RIF Ultra has an important role in TB diagnosis because it can rapidly detect Mycobacterium tuberculosis and rifampicin resistance.[3,4] In addition to detection results, this test produces cycle threshold (Ct) values and semiquantitative categories that reflect bacillary burden in the specimen.[5,6] A lower Ct value indicates a higher amount of bacillary genetic material in the specimen.[6] Previous evidence also supports the clinical use of Xpert Ct or semiquantitative categories as indicators related to bacterial burden, smear status, disease severity, and transmission risk.[7,8]

The neutrophil-to-lymphocyte ratio (NLR) is a systemic inflammatory marker calculated from the ratio of neutrophils to lymphocytes in a complete blood count.[9] This parameter is inexpensive, easily available, and has been investigated as a supportive biomarker in pulmonary TB and other respiratory infections.[10] Several studies have assessed NLR or related leukocyte ratios for TB diagnosis, treatment-response monitoring, and prognostic evaluation.[11] However, NLR mainly reflects the host immune response and does not necessarily move in parallel with bacillary burden indicators measured directly from respiratory specimens.[13]

Previous studies have mostly assessed NLR in general pulmonary TB, pediatric TB, or post-treatment changes.[12,14] Data specifically linking NLR with Ct categories in patients with drug-resistant TB remain limited. This gap is important because patients with drug-resistant

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TB commonly undergo hematological and molecular examinations during the same clinical period. This study aimed to analyze the correlation between blood NLR and molecular test Ct category in patients with drug-resistant TB.

METHODS

Study design

This analytic observational study used a cross-sectional design. The study was conducted at the Laboratory of Dr. H. A. Rotinsulu Lung Hospital, Bandung, from January to November 2024. This design was selected because the NLR and Ct categories were obtained during the same examination period.

Data source and sampling procedures

Data were obtained from laboratory and clinical records of patients with drug-resistant TB. Samples were selected using purposive sampling. Inclusion criteria were patients aged 18 years or older, diagnosed with drug-resistant TB, and who had traceable, complete hematology and Xpert MTB/RIF Ultra results. Exclusion criteria were incomplete laboratory data, pregnancy, and having received treatment for more than one month at data recording. The final sample consisted of 29 patients, representing all eligible patients during the study period.

Variable of study

The main variables were NLR and the molecular test Ct category. NLR was treated as a numerical inflammatory marker and was also described as normal or elevated for descriptive purposes. The CT category was treated as an ordinal semiquantitative molecular variable consisting of high, medium, low, and very low categories.[2,5,6] No very low category was found in this study.

Measurement and instrument

NLR was calculated by dividing the neutrophil percentage by the lymphocyte percentage from differential leukocyte counts. Hematological examination used a Sysmex XN-450 hematology analyzer based on fluorescent flow cytometry. Molecular testing used Xpert MTB/RIF Ultra. Ct category followed the semiquantitative interpretation of the molecular rapid test: high for Ct <16, medium for Ct 16-22, low for Ct 22-28, and very low for Ct >28 [2,5,6]. For additional description, this study classified NLR as normal (2.0-2.9) and elevated (>=3.2) based on the study data.

Data collection

Data were collected by tracing laboratory results of patients who met the criteria. Each subject was recorded by sex, NLR category, Ct category, and mean NLR according to Ct category.

Ethical consideration

This study received ethical approval from the Health Research Ethics Committee of Dr. H. A. Rotinsulu Lung Hospital with number LB.01.02/13524/2024. Subject identity was protected by using coded data.

Data analysis

Descriptive analysis was used to describe subject characteristics, NLR category distribution, Ct category distribution, and mean NLR. The Shapiro-Wilk test was used to assess data normality. Because one group was not normally distributed and the number of samples in the medium category was very small, NLR differences across Ct categories were analyzed using the Kruskal-Wallis test. The correlation between NLR and Ct category was

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analyzed using Spearman's test because Ct category is an ordinal semiquantitative variable. A p-value of <0.05 was set as the threshold for statistical significance.

RESULTS

Subject characteristics are presented in Table 1. Of 29 patients with drug-resistant TB, 19 (66%) were male, and 10 (34%) were female. Twenty-two patients (76%) had elevated NLR, whereas 7 (24%) had normal NLR. The Ct category was dominated by high in 15 patients (50%), followed by low in 11 patients (40%), and medium in 3 patients (10%).

Table 1. Characteristics of patients with drug-resistant tuberculosis

Characteristic	Category	n	%
Sex	Male	19	66
	Female	10	34
NLR category	Normal (2.0–2.9)	7	24
	Elevated (≥3.2)	22	76
Molecular test Ct category	High	15	50
	Medium	3	10
	Low	11	40

Mean NLR according to Ct category is shown in Table 2. The high Ct group had the highest mean NLR, at 6.76. The medium Ct group had a mean NLR of 3.50, whereas the low Ct group had a mean NLR of 5.49. Descriptively, this pattern did not form a consistent linear gradient across Ct categories.

Table 2. Mean neutrophil-to-lymphocyte ratio by Ct category

Molecular test Ct category	n	Mean NLR
High	15	6.76
Medium	3	3.50
Low	11	5.49

Spearman correlation analysis showed a correlation coefficient of 0.101 with a p-value of 0.300. This result indicates a very weak positive and statistically nonsignificant association between NLR and Ct category in patients with drug-resistant TB.

Table 3. Spearman correlation between NLR and Ct category

Variable	Spearman r	p-value	Interpretation
NLR vs molecular test Ct category	0.101	0.300	Very weak correlation; not significant

Differences in NLR values across Ct categories were analyzed using the Kruskal-Wallis test. The test showed H=1.667 with p=0.435. Therefore, there was no significant difference in NLR between high, medium, and low Ct groups.

Table 4. Kruskal-Wallis test of NLR differences across Ct categories

Compared groups	Statistical test	H	df	p-value	Interpretation
NLR across high, medium, and low Ct categories	Kruskal-Wallis	1.667	2	0.435	No significant difference

DISCUSSION

Interpretation of the main findings

The results showed that NLR was not significantly correlated with Ct category in patients with drug-resistant TB. The observed positive correlation was very weak, indicating that NLR changes were almost not aligned with shifts in Ct category. The comparative test also

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showed no significant NLR difference across Ct categories. This finding strengthens the interpretation that NLR mainly reflects the host systemic inflammatory response rather than the amount of bacillary DNA detected in sputum. [9,13]

Biologically, this result can be understood because Ct and NLR measure two different aspects. Ct is related to the amount of Mycobacterium tuberculosis genetic material in sputum specimens.[6,7] In contrast, NLR is influenced by systemic immune response, nutritional status, coinfection, metabolic comorbidity, inflammatory stress, and disease phase. In patients with drug-resistant TB, variation in disease history, treatment history, and clinical condition before testing may obscure the direct relationship between peripheral inflammation and semiquantitative molecular parameters. [9,13,14]

Comparison with previous studies

This finding is consistent with previous evidence on the position of NLR. Omair et al. (2024) showed that NLR, monocyte-to-lymphocyte ratio, and neutrophil-to-monocyte ratio were more relevant for monitoring treatment response during the intensive phase of pulmonary TB than for directly representing bacillary burden.[15] Suryana et al. (2022) reported that high pretreatment NLR was associated with delayed sputum conversion in bivariate analysis, but this factor did not always remain significant after multivariate analysis.[12] Kim et al. (2025) reported the real-world clinical utility of Xpert MTB/RIF Ultra in TB assessment and positioned molecular testing as a direct bacteriological tool.[16] Thus, NLR is more appropriately understood as a clinical and inflammatory risk marker, not as a substitute for a molecular indicator at a single examination point.

Fritschi et al. (2023) concluded that ratios derived from complete blood counts may support TB diagnosis, monitoring, and prognosis, but their performance varies by population, intended use, and clinical comparator.[13] Kissling et al. (2023) reported the potential value of monocyte, lymphocyte, and neutrophil ratios as easy-to-use biomarkers for pediatric TB diagnosis.[17] Djiu et al. (2025) used NLR to distinguish pulmonary TB from community-acquired pneumonia in children.[18] Regina et al. (2024) used NLR in the context of MDR-TB sputum conversion after three months of treatment.[19] Cursi et al. (2023) also examined leukocyte-derived ratios in children with active TB through a multicentre study.[20] The present study adds to this evidence by showing that, in patients with drug-resistant TB, NLR cannot be assumed to be equivalent to Ct category as a reflection of sputum bacillary burden.

Evidence on Xpert MTB/RIF Ultra also supports the need to maintain molecular examination as the main bacteriological assessment. Kim et al. (2025) described the real-world clinical utility of Xpert MTB/RIF Ultra in TB assessment in a low-incidence, high-resource setting.[16] Horne et al. (2025) reviewed Xpert MTB/RIF Ultra for pulmonary TB and rifampicin resistance in adults and adolescents.[21] Hueda-Zavaleta et al. (2024) reported diagnostic accuracy of Xpert MTB/RIF and Xpert Ultra compared with Lowenstein-Jensen culture.[22] These studies support the interpretation that hematological ratios may complement, but should not replace, molecular bacteriological testing.

Clinical implications, strengths, and limitations

The clinical implication of this study is that NLR may be considered an adjunct inflammatory marker in the general evaluation of patients with drug-resistant TB. Evidence from pulmonary TB studies also places NLR and related ratios as accessible markers for diagnosis, monitoring, or prognosis, although their performance varies across populations and clinical objectives.[23,24] Nutritional scores may help contextualize prognosis in pulmonary

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TB.[25] A systematic review also supports the role of related monocyte-to-lymphocyte ratios for TB diagnosis and monitoring. However, NLR is not appropriate as a replacement for molecular bacteriological parameters such as Xpert MTB/RIF Ultra Ct category [6,16,21]

The strength of this study lies in its focus on the drug-resistant TB population and the use of hematological and molecular data obtained during the same examination period. The limitations of this study include a small sample size, unequal Ct category distribution, very few subjects in the medium category, a cross-sectional design, and the absence of analytic control for confounding factors such as age, comorbidity, nutritional status, and treatment phase. Future studies should use multicenter designs, larger samples, more detailed comorbidity recording, and multivariate analysis to explain the clinical role of NLR in drug-resistant TB more robustly.

CONCLUSION

NLR did not show a significant correlation with Ct category in patients with drug-resistant TB. NLR also did not differ significantly across high, medium, and low Ct groups. These results indicate that NLR is more appropriately used as an adjunct inflammatory marker and cannot replace molecular bacteriological examination. Studies with larger samples and control of confounding factors are needed to clarify the clinical value of NLR in monitoring patients with drug-resistant TB.

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