



Original Research

The Relationship Between Widal Agglutination Titer Categories and Neutrophil-to-Lymphocyte Ratio in Patients with Acute Fever

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ABSTRACT

Background: Acute fever is a common clinical condition caused by various infectious agents, including *Salmonella Typhi*. The Widal test is widely used to support typhoid diagnosis because it is simple, rapid, and inexpensive, although its diagnostic accuracy is limited. The Neutrophil-Lymphocyte Ratio (NLR) is a hematological parameter reflecting systemic inflammation. This study aimed to determine the relationship between Widal agglutination titer categories and NLR in patients with fever

Methods : This observational analytical study used a cross-sectional approach. Secondary data were obtained from medical records and laboratory results. A total of 134 patients with fever were selected using purposive sampling. Widal titers and neutrophil and lymphocyte counts were recorded, and NLR was calculated by dividing the neutrophil count by the lymphocyte count. Data were analyzed using Spearman's correlation test at 0.05.

Results: Moderately positive Widal titers (1:160) predominated, accounting for 29.9% of patients. Low NLR values (<3) predominated at 44.0%. Spearman analysis showed a significant positive relationship between Widal titer categories and NLR ($p = 0.030$; $r = 0.187$), with a very weak correlation.

Conclusion: There was a significant positive relationship between Widal titers and NLR. However, the very weak correlation indicates that other factors may influence NLR values.

ARTICLE HISTORY

Submitted : 16-8-2026

Published : 01-10-2026

KEYWORDS

Acute Fever; Widal Titer;
Neutrophil-Lymphocyte Ratio
(NLR)

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Cite this as: Anak Agung Istri Tika Ulundia, Anak Agung Ayu Eka Cahyani, Moh. Fairuz Abadi, Diah Prihatiningsih(2026). The Relationship Between Widal Agglutination Titer Categories and Neutrophil-to-Lymphocyte Ratio in Patients with Acute Fever, 3(1). <https://doi.org/10.70920/jahns.v3i1.457>

INTRODUCTION

Acute fever is a common clinical condition that may be caused by various infectious agents, including *Salmonella enterica* serovar Typhi, the causative agent of typhoid fever. Typhoid fever remains an important infectious disease, particularly in developing countries, and early diagnosis can be challenging because its clinical manifestations are often nonspecific and may overlap with other acute febrile illnesses. Blood culture is an important reference method for confirming *S. Typhi* infection; however, its application may be limited by cost, laboratory infrastructure, technical requirements, and turnaround time. Therefore, serological tests such as the Widal agglutination test continue to be used in many healthcare facilities because they are relatively simple, inexpensive, and readily available

(Mawazo et al., 2019; Centers for Disease Control and Prevention [CDC], 2024). However, the interpretation of Widal results requires caution because diagnostic performance may vary according to endemicity, testing methods, previous exposure, and cross-reactivity (Mawazo et al., 2019; Najib et al., 2021).

Previous studies have demonstrated important limitations of the Widal test. Mawazo et al. (2019) reported that the Widal test showed poor agreement with blood culture and produced considerable false-positive and false-negative results. A Bayesian latent-class network analysis involving numerous typhoid diagnostic comparisons also demonstrated substantial variation in the sensitivity and specificity of Widal testing depending on antigen, titer, population, and geographic setting (Pastoor et al., 2019). Furthermore, a systematic review and meta-analysis found considerable variability in the performance of immunodiagnostic tests for typhoid fever, emphasizing the need for complementary diagnostic approaches (Najib et al., 2021). In another systematic review and meta-analysis, Muhie et al. (2023) reported pooled Widal sensitivity of 80.8% and specificity of 53.0%, indicating a relatively poor positive predictive value. More recent diagnostic accuracy studies have continued to demonstrate that Widal performance is inferior to several alternative serological or rapid diagnostic methods, although its usefulness may vary according to the clinical and epidemiological setting (Kabir et al., 2023; Nganga & Mweu, 2025). These findings support the importance of interpreting Widal results together with other laboratory parameters rather than using the test as a single diagnostic determinant.

The Neutrophil-Lymphocyte Ratio (NLR) is a simple hematological biomarker calculated from peripheral blood neutrophil and lymphocyte counts. It reflects the interaction between innate and adaptive immune responses and has been increasingly investigated as an indicator of systemic inflammation (Buonacera et al., 2022). In patients with typhoid fever, NLR may provide additional information regarding inflammatory activity. An Indonesian study conducted at Siti Rahmah Islamic Hospital, Padang, evaluated NLR among patients with typhoid fever and demonstrated the potential usefulness of this parameter in describing inflammatory conditions (Anggraini et al., 2025). Similarly, Zerlinda et al. (2023) investigated NLR in relation to typhoid IgG/IgM results and demonstrated the relevance of NLR as an additional hematological parameter in patients suspected of typhoid fever. These findings indicate that NLR may complement serological examinations by providing information about the systemic inflammatory response that cannot be directly obtained from antibody titers alone.

Despite the increasing evidence concerning the limitations of Widal testing and the potential role of NLR, previous studies have generally evaluated these parameters separately or compared NLR with other serological methods. Limited evidence is available regarding the specific relationship between Widal agglutination titer categories and NLR among patients presenting with acute fever. This represents an important research gap because Widal titers primarily reflect the humoral antibody response to *Salmonella Typhi* antigens, whereas NLR reflects systemic inflammatory activity. Investigating these parameters simultaneously may therefore provide a more comprehensive understanding of the relationship between serological response and inflammatory status. The novelty of the present study lies in examining the relationship between Widal agglutination titer categories and NLR values specifically among patients with acute fever, rather than limiting the study population to patients with confirmed typhoid fever. This approach is particularly relevant in clinical settings where definitive diagnostic facilities such as blood culture may not always be readily available.

Therefore, this study aimed to determine the relationship between Widal agglutination titer categories and NLR values among patients with acute fever at Ganesha General

Hospital. The study hypothesized that higher Widal agglutination titer categories would be associated with higher NLR values.

MATERIALS AND METHOD

This study employed a quantitative observational analytical design with a cross-sectional approach using retrospective secondary data from medical records and laboratory results at Ganesha General Hospital, Gianyar, Bali. The study was conducted from November 2025 to March 2026 using patient data recorded from July to December 2025. The study population consisted of 200 patients with acute fever who underwent Widal and complete blood count examinations. A total of 134 patients were selected using purposive sampling based on predetermined inclusion and exclusion criteria.

The independent variable was the Widal agglutination titer category, classified as negative, low positive (1:80), moderate positive (1:160), and high positive (1:320). The dependent variable was the Neutrophil-Lymphocyte Ratio (NLR), calculated by dividing the absolute neutrophil count by the absolute lymphocyte count and categorized as low (<3), moderate (3–5), or high (>5). Data were collected from medical records and laboratory reports, verified, coded, and entered into a statistical database.

Univariate analysis was performed using frequency and percentage distributions. The relationship between Widal titer categories and NLR was analyzed using Spearman's rank correlation because both variables were ordinal. Statistical significance was set at $\alpha = 0.05$ with a 95% confidence level, and $p < 0.05$ was considered statistically significant. Patient confidentiality was maintained throughout the study.

RESULTS

The study included 134 patients with acute fever who fulfilled the predetermined eligibility criteria. The findings are presented in terms of the distribution of Widal agglutination titer categories and NLR values, followed by a correlation analysis to assess the relationship between these variables.

Table 1. Characteristics of Study Subjects

No.	Subject Characteristics	Number of Participants (n)	Percentage (%)
A	Age		
1	Adolescents: <20 years	39	29.1
2	Early adults: 20–40 years	54	40.3
3	Late adults: 41–59 years	28	20.9
4	Older adults: ≥ 60 years	13	9.7
	Total	134	100
B	Sex		
1	Male	61	45.5
2	Female	73	54.5
	Total	134	100

Table 1 shows the characteristics of the 134 study subjects based on age and sex. The largest proportion of participants was in the early adult age group (20–40 years), comprising 54 participants (40.3%), followed by adolescents aged <20 years with 39 participants (29.1%), late adults aged 41–59 years with 28 participants (20.9%), and older adults aged ≥ 60 years with 13 participants (9.7%). In terms of sex, female participants predominated, with 73 participants (54.5%), while male participants accounted for 61 participants (45.5%). Overall, the study population consisted of 134 participants, representing 100% of the study sample.

Table 2. Distribution of Widal Test Results among Patients with Acute Fever

Widal Test Result	Frequency (n)	Percentage (%)
Negative	33	24.6
Low positive (1:80)	32	23.9
Moderately positive (1:160)	40	29.9
Highly positive (1:320)	29	21.6
Total	134	100

Table 2 presents the distribution of Widal test results among 134 patients with acute fever. The largest proportion of patients had a moderately positive Widal titer (1:160), comprising 40 patients (29.9%). This was followed by negative results in 33 patients (24.6%), low-positive titers (1:80) in 32 patients (23.9%), and high-positive titers (1:320) in 29 patients (21.6%). Overall, the findings indicate that the moderately positive Widal titer was the most frequently observed result among the study participants.

Table 3. Distribution of Neutrophil-Lymphocyte Ratio (NLR) among Patients with Acute Fever

NLR Category	Frequency (n)	Percentage (%)
Low (<3)	59	44.0
Moderate (3–5)	50	37.3
High (>5)	25	18.7
Total	134	100

Table 3 shows the distribution of the Neutrophil-Lymphocyte Ratio (NLR) among 134 patients with acute fever. The majority of patients had a low NLR category (<3), comprising 59 patients (44.0%). This was followed by the moderate NLR category (3–5), which included 50 patients (37.3%), while 25 patients (18.7%) had a high NLR category (>5). Overall, the findings indicate that the low NLR category was the most prevalent among the study participants.

Table 4. Spearman Correlation Test between Widal Titer and NLR

Variable	Correlation Coefficient (r)	p-value	N
Widal–NLR	0.187	0.030	134

Table 4 show the results of the Spearman correlation analysis between Widal agglutination titer categories and Neutrophil-Lymphocyte Ratio (NLR) among 134 patients with acute fever. The analysis showed a positive correlation coefficient of 0.187 with a *p*-value of 0.030. Since the *p*-value was less than 0.05, there was a statistically significant relationship between Widal titer categories and NLR. The positive correlation indicates that higher Widal titer categories tended to be associated with higher NLR values. However, the correlation coefficient of 0.187 indicates that the strength of the relationship was very weak, suggesting that other factors may also contribute to variations in NLR among patients with acute fever.

DISCUSSION

The present study demonstrates that Widal agglutination titers and the Neutrophil-Lymphocyte Ratio (NLR) were significantly associated among patients with acute fever, although the strength of the relationship was very weak. This finding suggests that an increase in Widal titer category tends to occur together with an increase in NLR, supporting the hypothesis that serological response and systemic inflammatory activity may have a directional relationship. However, the low correlation coefficient indicates that Widal titer alone does not adequately explain variations in NLR. This interpretation is consistent with the

biological characteristics of the two parameters: Widal testing reflects an antibody-mediated response to *Salmonella Typhi* antigens, whereas NLR represents the balance between neutrophil-mediated innate immunity and lymphocyte-mediated adaptive immunity and is therefore influenced by a broader range of inflammatory processes (Buonacera et al., 2022). The predominance of moderately positive Widal titers in this study should be interpreted cautiously. Although higher titers may indicate a stronger serological response, a positive Widal result cannot be considered definitive evidence of active typhoid infection. Previous research has demonstrated substantial variability in the diagnostic performance of Widal testing. Mawazo et al. (2019) found limited agreement between Widal results and culture-based diagnosis, while Muhie et al. (2023) reported pooled sensitivity of 80.8% but specificity of only 53.0%. More recent evidence also indicates that Widal performance varies according to population, epidemiological setting, and testing conditions. Kabir et al. (2023) reported considerable variation among commercially available typhoid diagnostic tests, while Nganga and Mweu (2025) found a sensitivity of 59.5% for Widal in symptomatic adults in Nairobi. Therefore, the Widal distribution observed in the present study should be regarded as a pattern of serological reactivity rather than a direct measure of confirmed *S. Typhi* infection.

The distribution of NLR, which was predominantly within the low and moderate categories, also provides an important context for interpreting the correlation. NLR is a nonspecific inflammatory biomarker and may be influenced by bacterial or viral infection, inflammatory conditions, physiological stress, medication use, and other clinical factors. Buonacera et al. (2022) emphasized that NLR reflects the interaction between innate and adaptive immune responses, but its interpretation requires consideration of the underlying clinical condition. Research specifically involving typhoid fever has also demonstrated the potential value of NLR as an indicator of inflammatory status. Anggraini et al. (2025), for example, described NLR among patients with typhoid fever at Siti Rahmah Islamic Hospital, Padang, supporting its potential use as an additional hematological parameter. Nevertheless, NLR should not be interpreted as a specific marker of typhoid infection because changes in neutrophil and lymphocyte counts may occur in many acute clinical conditions.

The statistically significant but very weak correlation found in this study is clinically meaningful because it demonstrates that Widal titer and NLR provide related but different information. The significant *p*-value indicates that the observed association is unlikely to have occurred solely by chance within this study population; however, the correlation coefficient indicates that the magnitude of the association is limited. This finding is also compatible with previous research showing that serological and inflammatory parameters do not necessarily demonstrate a strong correspondence. Zerlinda et al. (2023), for example, found no significant association between typhoid IgG/IgM results and NLR in their study of typhoid patients. The difference between that finding and the present study may be related to differences in the diagnostic method, study population, sample size, disease characteristics, and categorization of laboratory results. Thus, the present findings support the use of Widal and NLR as complementary rather than interchangeable parameters.

From a theoretical perspective, the findings support the concept that humoral immune responses and systemic inflammatory responses may occur concurrently during acute infectious processes but are not necessarily proportional. From a practical perspective, NLR may provide additional information when clinicians interpret Widal results, particularly in settings where definitive diagnostic methods are not readily available. However, the findings do not support the use of NLR as a substitute for confirmatory testing. Current evidence continues to emphasize the limitations of Widal testing and the need for more accurate diagnostic approaches. Systematic reviews have reported considerable variability in the performance of immunodiagnostic tests, while recent evidence continues to highlight the

need for improved typhoid diagnostics. Therefore, clinical symptoms, epidemiological information, hematological findings, and available confirmatory tests should be considered together when evaluating patients with suspected typhoid fever.

Several limitations should be considered when interpreting these findings. First, the study relied on Widal testing without blood culture or another definitive reference method; therefore, the observed relationship should not be interpreted as evidence that all patients with positive Widal titers had active *S. Typhi* infection. Second, the study used retrospective secondary data, limiting control over potentially important confounding factors such as duration of fever, previous antibiotic use, nutritional status, previous exposure to *Salmonella*, and other concurrent infections. Third, the categorization of both Widal titers and NLR into ordinal groups may reduce the amount of information available compared with analysis using continuous laboratory values. These limitations may partly explain the very weak correlation observed. Consequently, the findings should be interpreted as evidence of an association rather than a causal relationship.

Overall, the study indicates that Widal agglutination titer categories are significantly associated with NLR among patients with acute fever, but the relationship is very weak. The findings therefore provide support for a complementary interpretation of serological and hematological parameters rather than reliance on either parameter alone. Future studies using larger samples, continuous laboratory measurements, and a definitive reference method such as blood culture are needed to determine whether the observed association remains consistent in patients with microbiologically confirmed typhoid fever.

CONCLUSION

This study concludes that there was a statistically significant positive relationship between Widal agglutination titer categories and the Neutrophil-Lymphocyte Ratio (NLR) among patients with acute fever at Ganesha General Hospital. The correlation was positive but very weak ($r = 0.187$; $p = 0.030$), indicating that higher Widal titer categories tended to be accompanied by higher NLR values. However, the weak correlation suggests that NLR is influenced by factors beyond Widal titer and should therefore be interpreted as a complementary hematological parameter rather than an independent indicator of typhoid infection. These findings support the complementary use of Widal results and NLR in the laboratory assessment of patients with acute fever. Future studies should consider larger samples, additional clinical variables, and confirmatory diagnostic methods such as blood culture to provide stronger evidence regarding the relationship between Widal titers and NLR.

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