



CLINICAL AND HEMATOLOGICAL CORRELATES OF TUBEX-TF ANTI-O9 IGM REACTIVITY IN CHILDREN WITH TYPHOID FEVER

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ABSTRACT

Typhoid fever is a major pediatric health burden, yet the biological significance of varying semiquantitative TUBEX-TF reactivity levels remains unclear. To investigate the relationship between clinical manifestations, routine hematological parameters, and TUBEX-TF reactivity in pediatric typhoid fever. This cross-sectional study utilized medical records of children aged 0–18 years diagnosed with typhoid fever at Haji Adam Malik General Hospital, Medan, from 2023 to 2024. This study used purposive sampling, and included 142 samples. Patients were then stratified into TUBEX-TF score 4 and 6 groups. Data were analyzed using Chi-square, Fisher's exact, and Mann-Whitney U tests. The study included 142 children (mean age 10.8 years; 55.6% male), with 84 (59.2%) having a score of 6. No significant differences were found between the score groups regarding clinical manifestations, hemoglobin, leukocyte or platelet counts, and most leukocyte differentials. However, lymphocyte percentage was significantly higher in the score 6 group compared to the score 4 group ($26.76 \pm 17.91\%$ vs. $20.57 \pm 16.05\%$; $p=0.031$). Higher TUBEX-TF reactivity did not correspond with differences in clinical manifestations or most routine blood parameters.

Keywords: children; hematological parameters; lymphocytes; TUBEX-TF; typhoid fever

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INTRODUCTION

Typhoid fever, a systemic infection caused by *Salmonella enterica* serovar Typhi, continues to impose a substantial health burden in regions where access to safe water, sanitation, and microbiological diagnostics remains uneven. Contemporary global estimates demonstrate a considerable burden in South and Southeast Asia, with children representing an important affected population (Tipah et al., 2026). Indonesia remains an endemic setting, making diagnostic approaches that are rapid, accessible, and clinically interpretable particularly relevant (Hartanto, 2021).

Diagnostic uncertainty in enteric fever has clinically important consequences. Delayed recognition may allow progression to gastrointestinal haemorrhage, intestinal perforation, encephalopathy, sepsis, and death, whereas indiscriminate empirical treatment contributes to unnecessary antimicrobial exposure (Shafqat et al., 2025). Although blood culture remains the conventional reference standard, its diagnostic yield depends on specimen volume, timing of collection, and antecedent antimicrobial therapy; culture-based diagnosis also requires laboratory infrastructure and time (Liu et al., 2024).

TUBEX-TF is a rapid inhibition magnetic-binding immunoassay designed to detect antibodies directed against the O9 antigen of *S. Typhi*. Unlike a purely dichotomous serological test, it yields a semiquantitative colour score, with increasing values reflecting greater assay reactivity (Nurmawati et al., 2024). Recent Indonesian data have nevertheless shown that TUBEX-TF performance in routine practice is imperfect and varies according to the cutoff used, underscoring the need for careful clinical interpretation (Nurmawati et al., 2024).

The hematological phenotype of typhoid fever is heterogeneous and may include anaemia, leukopenia or leukocytosis, thrombocytopenia, and alterations in leukocyte differentials (Etouke et al., 2023). These abnormalities reflect multiple processes, including systemic inflammation, reticuloendothelial activation, marrow suppression, cellular redistribution, and disease phase. Because the complete blood count is inexpensive and almost universally available, determining whether specific hematological features track with anti-O9 IgM reactivity could provide mechanistic insight and identify clinically testable signals.

Evidence directly relating routine hematological parameters to the magnitude of semiquantitative TUBEX-TF reactivity remains limited. A previous Indonesian study evaluated hematological parameters across the TUBEX-TF colour scale and reported no uniform relationship across routine indices (Nugraheni et al., 2022). The present study therefore investigated whether clinical manifestations and routinely measured hematological parameters differed between children with TUBEX-TF scores of 4 and 6.

METHOD

This observational analytic study used a cross-sectional design and secondary medical-record data from Haji Adam Malik General Hospital, Medan, Indonesia. Medical records of paediatric patients evaluated for typhoid fever during 2023–2024 were reviewed. The study was conducted after approval by the Health Research Ethics Committee of Universitas Sumatera Utara and institutional research permission. The ethics approval number will be inserted in the final submission file after verification against the original ethics document.

The study population comprised children aged 0–18 years with clinically suspected typhoid fever. Eligible subjects had fever or a history of fever accompanied by gastrointestinal manifestations such as nausea/vomiting, diarrhoea, or constipation and had undergone both complete blood count and TUBEX-TF testing. Patients were excluded if they had chronic hematological or congenital blood disorders, severe chronic diseases capable of altering hematological parameters, or incomplete or unreliable hematological data. The calculated minimum sample size was 132 subjects; 142 eligible records were included in the final analysis.

Clinical variables extracted from medical records included constipation, diarrhoea, nausea/vomiting, duration of fever, and other recorded manifestations. Hematological variables included haemoglobin concentration, total leukocyte count, platelet count, and differential percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

TUBEX-TF testing was performed using the commercial TUBEX-TF kit (IDL Biotech, Sweden). For the assay, 45 µL of brown reagent and 45 µL of patient serum were incubated for 2 minutes, followed by the addition of 90 µL of blue reagent. After shaking for 2 minutes, the reaction was compared with the TUBEX-TF colour scale after 5 minutes. Scores range from 0 to 10, with a score of 4 or greater interpreted as positive according to the manufacturer. In the present dataset, positive results were classified as score 4 or score 6 (Nurmawati et al., 2024).

Data were then analysed. Categorical variables were summarised as frequencies and percentages. Numerical variables were reported as mean $\pm$ standard deviation to remain consistent with the source dataset and study tables. Normality was assessed using the Shapiro-Wilk test. Because most numerical variables were non-normally distributed, between-group comparisons were performed using the Mann-Whitney U test. Categorical variables were compared using the chi-square test or Fisher exact test when appropriate. A two-sided p value <0.05 was considered statistically significant.

RESULT

A total of 142 children met the eligibility criteria. Mean age was 10.8 ± 6.2 years, and 79 (55.6%) were male. TUBEX-TF score 6 was observed in 84 children (59.2%), whereas 58 (40.8%) had score 4. Fever was recorded in all subjects. Nausea/vomiting was the most frequent gastrointestinal manifestation (63.4%), followed by diarrhea (38.0%) and constipation (13.4%). Mean fever duration was 10.6 ± 14.1 days (Table 1).

Table 1.
Characteristics of the study participants

Characteristics	Total (N=142)
Age, mean $\pm$ SD, years	10.8 ± 6.2
Male, n (%)	79 (55.6)
Female, n (%)	63 (44.4)
TUBEX-TF score 4, n (%)	58 (40.8)
TUBEX-TF score 6, n (%)	84 (59.2)
Constipation, n (%)	19 (13.4)
Diarrhoea, n (%)	54 (38.0)
Nausea/vomiting, n (%)	90 (63.4)
Fever duration, mean $\pm$ SD, days	10.6 ± 14.1

None of the evaluated clinical manifestations differed significantly between TUBEX-TF score groups. Constipation ($p=0.166$), diarrhea ($p=0.282$), nausea/vomiting ($p=0.328$), and fever duration ($p=0.735$) were not associated with semiquantitative TUBEX-TF reactivity (Table 2).

Table 2.
Relationship between clinical manifestations and TUBEX-TF score

Clinical variable	TUBEX-TF 4	TUBEX-TF 6	p-value
Constipation, yes/no	5 / 53	14 / 70	0.166 ^a
Diarrhoea, yes/no	19 / 39	35 / 49	0.282 ^a
Nausea/vomiting, yes/no	34 / 24	56 / 28	0.328 ^a
Fever duration	-	-	0.735 ^b

^aChi-square; ^bNumerical variable

Lymphocyte percentage was the only hematological parameter that differed significantly between TUBEX-TF score groups. Mean lymphocyte percentage was higher in score 6 than score 4 ($26.76 \pm 17.91\%$ vs $20.57 \pm 16.05\%$; $p=0.031$). Neutrophil percentage tended to be lower in score 6, but the difference did not reach statistical significance ($p=0.082$). No significant differences were observed for hemoglobin, total leukocyte count, platelet count, basophils, eosinophils, or monocytes (Table 3).

Table 3.
Hematological parameters according to TUBEX-TF score

Parameter	TUBEX-TF 4 Mean $\pm$ SD	TUBEX-TF 6 Mean $\pm$ SD	p value
Hemoglobin, g/dL	11.54 ± 2.37	11.14 ± 1.91	0.193 ^a
Leukocytes, /mm ³	$10,040 \pm 7,015$	$11,468 \pm 19,246$	0.575 ^a
Platelets, /mm ³	$262,054 \pm 187,389$	$243,878 \pm 165,228$	0.763 ^a
Neutrophils, %	53.44 ± 30.75	47.87 ± 27.69	0.082 ^a
Basophils, %	0.31 ± 0.36	0.32 ± 0.46	0.536 ^a
Lymphocytes, %	20.57 ± 16.05	26.76 ± 17.91	0.031 ^a
Eosinophils, %	11.03 ± 25.00	11.50 ± 24.69	0.322 ^a
Monocytes, %	13.47 ± 16.32	12.54 ± 10.44	0.229 ^a

^aMann-Whitney U test

DISCUSSION

The principal finding of this study is that increasing semiquantitative anti-O9 IgM reactivity was not accompanied by a parallel shift in the conventional clinical or hematological phenotype of paediatric typhoid fever. Instead, lymphocyte percentage emerged as the sole parameter associated with higher TUBEX-TF reactivity. This pattern suggests that routine hematological disturbances do not simply scale with serological intensity and is consistent with the fact that TUBEX-TF reflects anti-O9 IgM reactivity rather than a direct measure of bacterial burden or disease severity

(Nurmawati et al., 2024).

The mean age of the study population was 10.8 years, consistent with the recognised burden of enteric fever among school-aged children (Enkelmann et al., 2025). Increasing independence, food consumption outside the home, and environmental exposure may increase opportunities for faecal-oral transmission. The broad age range in this cohort, however, also introduces biological heterogeneity because immune maturation and age-dependent leukocyte distributions vary throughout childhood.

Gastrointestinal manifestations were common, particularly nausea/vomiting and diarrhoea, yet none of the evaluated clinical features was associated with TUBEX-TF score. Paediatric enteric fever has a heterogeneous clinical spectrum, and the frequency of specific manifestations varies with age and setting (Enkelmann et al., 2025). Clinical symptoms reflect the integrated effects of intestinal invasion, local and systemic inflammation, host response, disease phase, and complications, whereas TUBEX-TF captures a specific component of the humoral response. Symptom frequency would therefore not be expected to increase uniformly with anti-O9 IgM reactivity.

The absence of an association between fever duration and TUBEX-TF score further illustrates this distinction. Antibody kinetics are influenced by the timing of infection, host immune response, and prior treatment rather than by reported fever duration alone. Indonesian prospective data have also demonstrated variation in TUBEX-TF positivity according to illness day and cutoff, reinforcing the importance of timing when interpreting serological results (Nurmawati et al., 2024).

Haemoglobin did not differ significantly between TUBEX-TF score groups. Anaemia in typhoid fever is multifactorial and may reflect inflammation-mediated iron restriction, reticuloendothelial activation, marrow suppression, haemophagocytosis, nutritional status, pre-existing iron stores, or gastrointestinal blood loss (Enkelmann et al., 2025; Ringoringo et al., 2022). These processes operate on different timescales from an acute serological response. Because iron-status biomarkers were unavailable, subclinical iron restriction could not be evaluated in the present study.

Among the hematological findings, the leukocyte differential provides the most biologically compelling signal. Neutrophil percentage showed a nonsignificant tendency to decrease with greater TUBEX-TF reactivity, whereas lymphocyte percentage was significantly higher in the score 6 group. *S. Typhi* is a facultative intracellular pathogen whose control requires coordinated innate and adaptive immunity. Antigen-specific cellular responses support intracellular bacterial control, while B-cell activation underpins the humoral response detected by serological assays (Tahir et al., 2024). Within this framework, the higher lymphocyte proportion observed at score 6 is compatible with, although does not prove, a relative predominance of adaptive immune activity accompanying stronger anti-O9 IgM reactivity.

This interpretation is biologically plausible because IgM is a characteristic early product of activated B-cell responses before selected clones undergo class-switch recombination to other immunoglobulin isotypes (Liu et al., 2024). Prospective studies integrating absolute lymphocyte counts, B- and T-cell immunophenotyping, quantitative anti-O9 antibody measurements, and serial sampling would help determine whether the signal observed here maps to a definable phase or cellular compartment of the adaptive response.

Eosinophil percentage, total leukocyte count, and platelet count were not associated with TUBEX-TF score. Typhoid fever may present with leukopenia, a normal leukocyte count, or leukocytosis, while platelet abnormalities may reflect marrow effects, splenic sequestration, peripheral consumption, or systemic inflammation (Etouke et al., 2023; Ringoringo et al., 2022). The lack of a uniform relationship with TUBEX-TF is therefore unsurprising. The absence of a significant

platelet association is also consistent with previous work comparing hematological parameters across the TUBEX-TF colour scale (Nugraheni et al., 2022).

Potential confounding should also be considered for eosinophil values. In endemic paediatric populations, helminth infection may increase eosinophil counts and thereby obscure infection-related eosinopenia; a recent large-scale cross-sectional study in Southeast Asia demonstrated a significant association between intestinal parasitic infections and elevated peripheral eosinophilia across pediatric and adult demographics (Webster et al., 2022). Because stool examination and allergic history were not systematically available, these factors could not be evaluated.

The findings should also be interpreted in light of the diagnostic limitations of TUBEX-TF itself. A recent multicentre Indonesian study found that TUBEX-TF showed variable sensitivity and specificity depending on the selected cutoff and could overdiagnose enteric fever when compared with culture and molecular confirmation (Nurmawati et al., 2024). Accordingly, the present analysis addresses correlates of TUBEX-TF reactivity among clinically diagnosed children rather than determinants of microbiologically confirmed *S. Typhi* infection.

Several limitations define the boundaries of inference. The retrospective, single-centre design relied on routine documentation and did not standardise sampling by illness day or systematically grade symptom severity. The wide paediatric age range introduces age-related variation in leukocyte differentials. Microbiological confirmation was not available for all subjects, and the dataset did not include absolute differential counts, lymphocyte immunophenotyping, quantitative IgM, or iron-status biomarkers. These limitations preclude causal or diagnostic claims. Nevertheless, the study exceeded its prespecified minimum sample size and simultaneously evaluated clinical manifestations and multiple routinely available hematological domains within a paediatric cohort.

Collectively, these findings argue against interpreting the TUBEX-TF colour score as a surrogate measure of the overall clinical or hematological intensity of typhoid fever. Rather, the assay appears to capture a dimension of host humoral reactivity that is only incompletely represented by routine peripheral-blood phenotypes. The selective lymphocyte signal is therefore of particular interest because it offers a testable link between a widely used rapid serological assay and the cellular architecture of adaptive immunity.

CONCLUSION

In this paediatric cohort, increasing TUBEX-TF anti-O9 IgM reactivity was not accompanied by corresponding differences in gastrointestinal manifestations or broad alterations in routine hematological indices. Lymphocyte percentage was the sole hematological parameter significantly associated with TUBEX-TF score and was higher among children with score 6. This selective relationship raises the possibility that semiquantitative serological reactivity may align more closely with specific components of the adaptive immune response than with nonspecific hematological manifestations of systemic inflammation. Because lymphocytes were assessed as relative percentages rather than absolute counts and no lymphocyte immunophenotyping was performed, this observation remains hypothesis-generating.

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