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Original Article

Diagnostic Accuracy of White Blood Cell Count, Serum Bilirubin, and C-Reactive Protein for Predicting Perforated Appendicitis: A Cross-Sectional Study

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Abstract

Background: Diagnosing perforated appendicitis remains a significant clinical challenge, particularly in resource-limited settings where access to advanced imaging modalities is restricted. Biochemical markers such as white blood cell (WBC) count, serum bilirubin, and C-reactive protein (CRP) are widely available and may provide a cost-effective means for early identification of complicated appendicitis.

Objective: To evaluate the diagnostic accuracy of WBC count, serum bilirubin, and CRP in identifying perforated appendicitis among patients presenting with suspected acute appendicitis.

Methodology: This descriptive cross-sectional diagnostic accuracy study was conducted at the Department of General Surgery, Liaquat University Hospital, Hyderabad. A total of 200 patients aged 18–40 years with clinical suspicion of acute appendicitis were enrolled. Blood samples were analyzed for WBC count (cells/µL), serum bilirubin (mg/dL), and CRP (mg/L) at presentation. Contrast-enhanced computed tomography (CT) was used as the reference standard. Diagnostic performance was assessed using sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operating characteristic (ROC) curve analysis.

Results: Among the 200 patients, 80 (40%) were diagnosed with perforated appendicitis. CRP demonstrated the highest diagnostic accuracy, with sensitivity of 78.75% and specificity of 88.33% (AUC: 0.87; 95% CI: 0.82–0.94). WBC count showed the highest sensitivity (85.00%) but lower specificity (73.33%) (AUC: 0.84; 95% CI: 0.78–0.89). Serum bilirubin demonstrated moderate sensitivity (76.25%) and lower specificity (53.33%) (AUC: 0.73; 95% CI: 0.66–0.81).

Conclusion: CRP, WBC count, and serum bilirubin are useful adjunctive biomarkers for predicting perforated appendicitis, with CRP demonstrating the highest overall diagnostic performance. These readily available and low-cost tests may serve as effective triage tools, particularly in settings where access to CT imaging is limited or delayed.

Keywords: C-reactive protein, Diagnostic accuracy, Perforated appendicitis, ROC curve, Serum bilirubin, WBC count.

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Introduction

Acute appendicitis is one of the most common causes of acute abdominal pain requiring emergency surgical intervention worldwide. Although diagnosis is often straightforward in patients with classical clinical presentations, atypical symptoms and delayed presentation can complicate clinical assessment, increasing the risk of disease progression to gangrene or perforation—conditions associated with higher morbidity, prolonged hospital stay, and increased healthcare costs [1-3].

Early and accurate identification of perforated appendicitis is critical, as it directly influences surgical decision-making, perioperative management, and patient outcomes. Imaging modalities such as ultrasonography and computed tomography (CT) have substantially improved diagnostic accuracy; however, their availability remains limited in many resource-constrained settings. Additionally, CT imaging is associated with increased cost and exposure to ionizing radiation, raising concerns about its routine use, particularly in younger populations [4]. Consequently, there is a continued need for simple, rapid, and cost-effective diagnostic tools that can aid in the early recognition of complicated appendicitis.

Routine laboratory parameters, including white blood cell (WBC) count and C-reactive protein (CRP), are widely used in the initial evaluation of suspected appendicitis. While leukocytosis is a common finding, its diagnostic utility in distinguishing uncomplicated from complicated appendicitis is limited due to relatively low specificity [5,6]. In contrast, CRP, an acute-phase reactant synthesized by the liver in response to systemic inflammation, has demonstrated a stronger association with disease severity, particularly in cases of appendiceal perforation or gangrene [7-9].

More recently, serum bilirubin has emerged as a potential biomarker for predicting appendiceal perforation. The proposed mechanism involves bacterial translocation and endotoxemia leading to hepatocellular dysfunction and impaired bilirubin excretion during severe inflammation [10]. Several studies have reported a significant association between hyperbilirubinemia and complicated appendicitis, with some suggesting higher specificity compared to traditional inflammatory markers [11-14]. For instance, Basukala et al. highlighted the diagnostic value of serum bilirubin in predicting perforation and emphasized its role in early clinical triage [14].

Despite growing evidence supporting the utility of WBC count, CRP, and serum bilirubin, their relative and combined diagnostic performance remains inconsistently reported across studies, with variability in cut-off values, study populations, and methodological approaches [8,9]. Furthermore, data from low- and middle-income countries remain limited, where reliance on accessible diagnostic tools is particularly important.

Therefore, the present study was designed to evaluate and compare the diagnostic accuracy of WBC count, serum bilirubin, and CRP in predicting perforated appendicitis among patients presenting with suspected acute appendicitis, using contrast-enhanced CT as the reference standard.

Methodology

Study Design

This study was designed as a descriptive cross-sectional diagnostic accuracy study conducted to evaluate the performance of biochemical markers in predicting perforated appendicitis. The study followed a structured diagnostic framework in which index tests (WBC count, serum bilirubin, and CRP) were compared against a predefined reference standard.

Ethics

Ethical approval was obtained from the Research Ethics Committee of Liaquat University Hospital (approval no. LUMHS/REC/-1303). The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment, ensuring voluntary participation, confidentiality, and the right to withdraw at any stage without affecting clinical care.

Setting

The study was carried out at the Department of General Surgery, Liaquat University Hospital, Hyderabad, a tertiary care center catering to both urban and rural populations. Data collection was conducted over a six-month period from 1 October 2024 to 31 March 2025, ensuring adequate patient recruitment and representation of routine clinical practice.

Participants

Consecutive adult patients aged 18–40 years presenting to emergency or outpatient surgical departments with clinical suspicion of acute appendicitis were assessed for eligibility. Clinical suspicion was based on right lower quadrant abdominal pain accompanied by symptoms such as

tenderness, fever, nausea, vomiting, or leukocytosis. The age restriction was intentionally applied to minimize biological variability in inflammatory responses, thereby enhancing internal validity. Patients were excluded if they refused consent, had incomplete laboratory or imaging data, or had conditions influencing inflammatory markers or bilirubin metabolism (e.g., chronic liver disease, viral hepatitis, hemolytic disorders, or biliary tract disease). Patients with diabetes mellitus or hypertension were included unless associated with hepatic dysfunction.

Variables

The primary outcome variable was the presence or absence of perforated appendicitis, as determined by contrast-enhanced computed tomography (CT). Independent variables included biochemical markers: total white blood cell (WBC) count (cells/μL), serum total bilirubin (mg/dL), and C-reactive protein (CRP) levels (mg/L). Additional covariates included demographic characteristics (age, gender, residential status) and clinical features (fever, nausea, vomiting, abdominal distension, and duration of symptoms).

Data Sources/Measurement

Blood samples for WBC count, serum bilirubin, and CRP were collected at initial presentation prior to imaging or surgical intervention, ensuring temporal consistency in biomarker measurement. Laboratory analyses were performed using standard hospital protocols. Contrast-enhanced CT was used as the reference standard for diagnosing appendiceal perforation, as histopathology was not universally available due to conservative management in some patients. Data were recorded using structured proformas to ensure completeness and uniformity.

Bias

Several measures were taken to minimize bias. Consecutive sampling reduced selection bias, while predefined inclusion and exclusion criteria limited confounding from comorbid conditions affecting biomarkers. However, potential sources of bias included the use of CT as the reference standard instead of histopathology (introducing possible misclassification bias), inclusion of non-appendicitis cases in the non-perforated group (affecting specificity estimates), and lack of standardization in the interval between symptom onset and blood sampling, which may influence biomarker levels. Additionally, restriction to a specific age group may limit external validity.

Study Size

The sample size was calculated using a diagnostic accuracy formula, assuming a prevalence of perforated appendicitis of 40%, expected sensitivity of serum bilirubin of 70.5%, specificity of 80.9%, a 95% confidence level, and 10% absolute precision. Based on these parameters, a minimum sample size of 200 patients was required, which was achieved in the study.

Quantitative Variables

Continuous variables such as WBC count, CRP, and serum bilirubin were analyzed both as continuous measures and categorized based on clinically established cut-off values: CRP >5 mg/L, WBC >11,000 cells/μL, and serum bilirubin >1.2 mg/dL. These thresholds were selected based on institutional laboratory reference ranges and supported by prior literature. Due to non-normal distribution of WBC and CRP (right-skewed), data were summarized using medians and interquartile ranges (IQR), while approximately normal variables were expressed as mean ± standard deviation.

Statistical Methods

Statistical analysis was performed using SPSS version 21.0. Normality of data was assessed using the Shapiro-Wilk test along with evaluation of skewness and kurtosis. Non-parametric tests were applied for group comparisons due to skewed distributions, with the Mann–Whitney U test used for continuous variables and chi-square test for categorical variables. Diagnostic accuracy was evaluated through sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy, with 95% confidence intervals calculated using the Wilson method. Receiver operating characteristic (ROC) curve analysis was performed to determine the discriminatory ability of each biomarker, and the area under the curve (AUC) was calculated.

Results

Participants

A total of 200 patients presenting with clinical suspicion of acute appendicitis were included in the final analysis. All enrolled participants completed the required laboratory and imaging assessments and were therefore included in the diagnostic accuracy evaluation. The study population represented a typical cohort of patients presenting to a tertiary care surgical unit, with no significant loss to follow-up or missing data reported.

Descriptive Data

The study population was relatively young, with a mean age of 28.53 ± 6.86 years, reflecting the selected inclusion criteria. There was a slight female predominance, with 106 (53.0%) females and 94 (47.0%) males. The majority of participants were from rural areas (69.0%), indicating the

hospital's catchment population. Clinically, most patients presented with classical symptoms of acute appendicitis, with fever (68.5%), nausea (63.5%), and vomiting (57.0%) being the most frequently reported symptoms, followed by abdominal distension (54.5%). Comorbid conditions such as diabetes mellitus (11.5%) and hypertension (14.0%) were present in a minority of patients. These baseline demographic and clinical characteristics are summarized in Table 1.

Outcome Data

Based on contrast-enhanced CT findings, patients were categorized into three groups: no appendicitis (21 patients; 10.5%), non-perforated appendicitis (99 patients; 49.5%), and perforated appendicitis (80 patients; 40.0%). For diagnostic accuracy analysis, the non-perforated group (n = 120) included both patients with uncomplicated appendicitis and those without appendicitis, reflecting real-world clinical decision-making scenarios. Patients with perforated appendicitis demonstrated significantly higher levels of all three biomarkers compared to the non-perforated group. Median serum bilirubin levels were 1.77 mg/dL (IQR: 1.23–2.04) in the perforated group versus 1.08 mg/dL (IQR: 0.85–1.66) in the non-perforated group. Similarly, median WBC count was elevated (14,097 vs 10,454 cells/μL), as was CRP (5.64 vs 4.10 mg/L), with all differences reaching strong statistical significance ($p < 0.001$). These comparisons are detailed in Table 2.

Main Results

All three biomarkers—CRP, WBC count, and serum bilirubin—demonstrated a statistically significant association with perforated appendicitis when analyzed using predefined clinical cut-off values ($p < 0.001$ for all comparisons; Table 3). Among these, CRP showed the highest overall diagnostic performance, with a sensitivity of 78.75% and a notably high specificity of 88.33%, resulting in an overall diagnostic accuracy of 84.50%. WBC count demonstrated the highest sensitivity (85.00%), indicating strong ability to detect perforation, although its specificity was comparatively lower (73.33%), suggesting reduced discriminative ability for ruling out disease. Serum bilirubin showed moderate sensitivity (76.25%) but relatively low specificity (53.33%), limiting its standalone diagnostic utility. These diagnostic indices are summarized in Table 4.

Receiver operating characteristic (ROC) curve analysis further confirmed the superior diagnostic performance of CRP, which achieved the highest area under the curve (AUC: 0.87; 95% CI: 0.82–0.94), indicating excellent discriminative ability. WBC count also demonstrated strong performance (AUC: 0.84; 95% CI: 0.78–0.89), while serum bilirubin showed comparatively lower accuracy (AUC: 0.73; 95% CI: 0.66–0.81). All biomarkers showed statistically significant AUC values ($p < 0.001$), confirming their ability to differentiate between perforated and non-perforated appendicitis. The ROC-derived optimal cut-off values were consistent with the predefined clinical thresholds. These findings are presented in Table 5, and the comparative ROC curves are illustrated in Figure 1, where CRP demonstrates the largest area under the curve, followed by WBC count and serum bilirubin.

Table 1: Demographics and Baseline Characteristics (n=200)

Variable	Category	Value (%)
Age (years)	—	28.53 ± 6.86
Gender	Male	94 (47.0)
	Female	106 (53.0)
Residential Status	Rural	138 (69.0)
	Urban	62 (31.0)
Diabetes Mellitus	Yes	23 (11.5)
Hypertension	No	172 (86.0)
	Yes	28 (14.0)
Fever	No	63 (31.5)
	Yes	137 (68.5)
Nausea	No	73 (36.5)
	Yes	127 (63.5)
Vomiting	No	86 (43.0)
	Yes	114 (57.0)
Abdominal Distension	No	91 (45.5)
	Yes	109 (54.5)

Table 2: Comparison of Biomarkers Between Perforated and Non-Perforated Appendicitis

Variable	Perforated (n = 80) Median (IQR)	Non-Perforated (n = 120) Median (IQR)	p-value*
Serum Bilirubin (mg/dL)	1.77 (1.23–2.04)	1.08 (0.85–1.66)	<0.001
WBC Count (cells/μL)	14,097 (11,620–16,738)	10,454 (9,321–12,039)	<0.001
CRP (mg/L)	5.64 (5.14–6.48)	4.10 (3.60–4.70)	<0.001

Mann–Whitney U test

Table 3: Diagnostic Accuracy of Biomarkers Using Predefined Clinical Cut-off Values (n = 200)

Biomarker	Category	Perforated (n = 80)	Non-Perforated (n = 120)	p-value*
CRP	>5 mg/L	63	14	<0.001
	≤5 mg/L	17	106	
WBC Count	>11,000 cells/μL	68	32	<0.001
	≤11,000 cells/μL	12	88	
Bilirubin	>1.2 mg/dL	61	56	<0.001
	≤1.2 mg/dL	19	64	

Chi-square test applied

Table 4: Diagnostic Performance of Biomarkers in Predicting Perforated Appendicitis

Biomarker	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy % (95% CI)
CRP >5 mg/L	78.75 (68.17–87.11)	88.33 (81.20–93.47)	81.82 (73.08–88.18)	86.18 (80.27–90.53)	84.50 (78.73–89.22)
WBC >11,000 cells/μL	85.00 (75.26–92.00)	73.33 (64.49–80.99)	68.00 (60.90–74.35)	88.00 (81.15–92.59)	78.00 (71.61–83.54)
Bilirubin >1.2 mg/dL	76.25 (65.42–85.05)	53.33 (44.01–62.49)	52.14 (46.47–57.75)	77.11 (68.73–83.77)	62.50 (55.39–69.23)

Table 5: Receiver Operating Characteristic (ROC) Analysis of Biomarkers

Biomarker	AUC (95% CI)	Standard Error	p-value	Optimal Cut-off	Sensitivity	1 – Specificity
Total Bilirubin	0.73 (0.66–0.81)	0.037	<0.001	1.19 mg/dL	0.75	0.46
WBC Count	0.84 (0.78–0.89)	0.031	<0.001	10,984.74 cells/μL	0.84	0.30
CRP Level	0.87 (0.82–0.94)	0.029	<0.001	5.04 mg/L	0.78	0.11

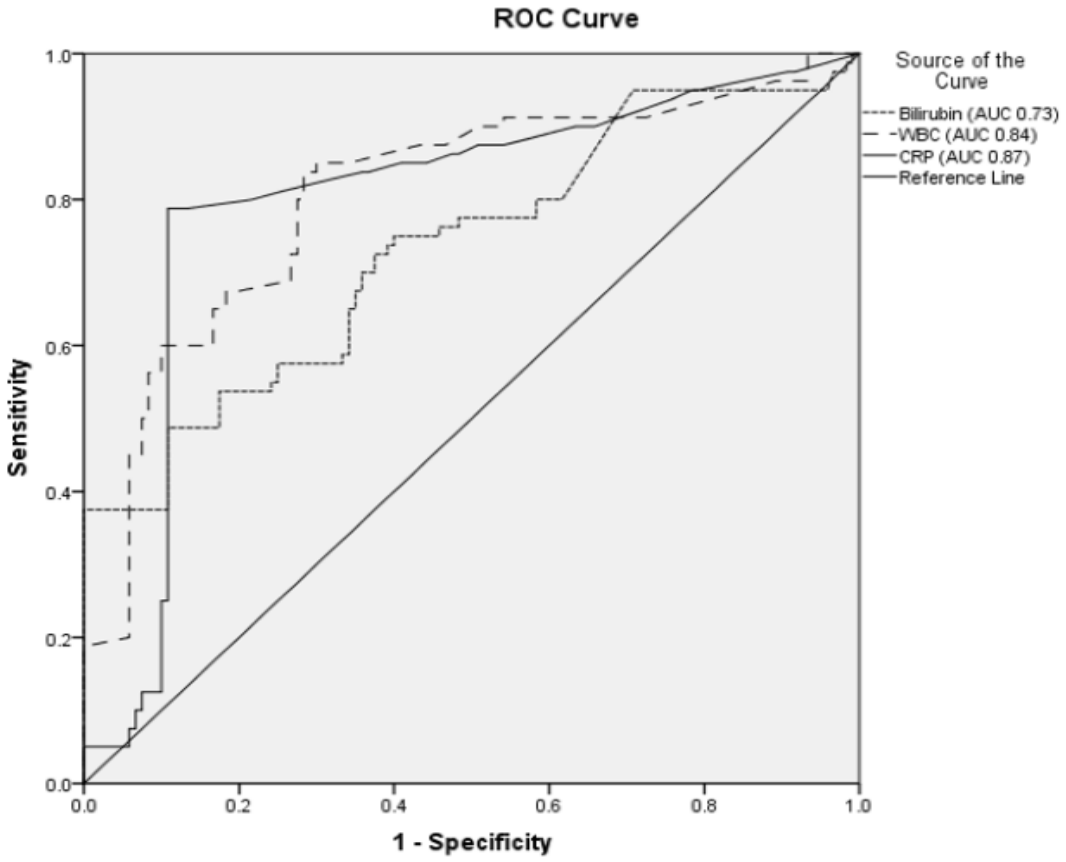


Figure 1: Receiver operating characteristic (ROC) curves for CRP, WBC count, and serum bilirubin in predicting perforated appendicitis. The diagonal gray line represents the reference line of no discriminative ability (AUC = 0.5). Solid line = CRP (AUC 0.87; 95% CI: 0.82–0.94); dashed line = WBC count (AUC 0.84; 95% CI: 0.78–0.89); dotted line = serum bilirubin (AUC 0.73; 95% CI: 0.66–0.81).

Discussion

Accurate and timely identification of perforated appendicitis remains a critical determinant of clinical outcomes, as delayed diagnosis is associated with increased morbidity, postoperative complications, and prolonged hospitalization. In the present study, commonly available biochemical markers—C-reactive protein (CRP), white blood cell (WBC) count, and serum bilirubin—demonstrated significant diagnostic utility in differentiating perforated from non-perforated appendicitis, with CRP showing the highest overall diagnostic accuracy.

CRP emerged as the most reliable biomarker in this study, exhibiting the highest area under the curve (AUC: 0.87) along with a favorable balance between sensitivity and specificity.[15] This finding is consistent with the established role of CRP as an acute-phase reactant that reflects the magnitude and duration of systemic inflammation. Its superior performance in detecting perforation likely reflects its strong correlation with disease severity and tissue damage. Similar observations have been reported by Patmano et al. [2] and Banerjee et al. [15], who demonstrated that elevated CRP levels are significantly associated with appendiceal perforation and can serve as an important tool for clinical risk stratification.

WBC count demonstrated relatively high sensitivity but lower specificity compared to CRP, indicating its usefulness as an initial screening marker but limited ability to distinguish between uncomplicated and complicated appendicitis. This aligns with findings from previous studies, including those by Emmanuel A et al. [16], Raza M et al. [17], Garg S et al. [18], and Anum T et al. [19], which highlight that leukocytosis is an early inflammatory response but lacks specificity for disease severity.

Serum bilirubin, although significantly associated with perforated appendicitis, showed comparatively lower diagnostic performance in this study. The pathophysiological basis of hyperbilirubinemia in

appendicitis is thought to involve bacterial endotoxemia leading to hepatocellular dysfunction and impaired bilirubin excretion [10]. This mechanism has been supported by studies such as those by Zosimas et al. [1], Ghosh et al. [3], Sreedevi et al. [11], Sen et al. [12], Doma et al. [13], and Basukala et al. [14]. However, the relatively lower specificity observed in our findings suggests that bilirubin alone may not be a reliable standalone diagnostic marker but may have adjunctive value when combined with other inflammatory parameters.

Importantly, the results of this study support a multimarker diagnostic approach. Previous research has demonstrated that combining CRP, WBC, and bilirubin improves overall diagnostic accuracy compared to individual markers alone. Studies by Sarkar et al. [20] and Sreeramulu and Shashidhar [21] reported enhanced sensitivity and specificity with combined biomarker use, while Ulusahin et al. [22] emphasized the importance of integrating laboratory findings with clinical assessment for improved diagnostic precision. These findings reinforce the clinical relevance of a composite diagnostic strategy, particularly in resource-limited settings.

The restriction of the study population to individuals aged 18–40 years was a deliberate methodological choice to reduce heterogeneity in inflammatory responses and improve internal validity. It is well documented that pediatric and geriatric populations exhibit different immune responses, atypical presentations, and altered baseline biomarker levels, which can significantly influence diagnostic accuracy.[22] While this approach strengthens the reliability of findings within the selected age group, it limits generalizability to broader populations.

The findings of this study highlight the practical value of readily available laboratory markers in the early identification of perforated appendicitis, particularly in settings where access to advanced imaging modalities is limited or delayed. CRP, in particular, demonstrates strong potential as a primary biomarker for risk stratification and clinical decision-making.

Limitations

Several limitations should be considered when interpreting the findings of this study. First, contrast-enhanced CT was used as the reference standard instead of histopathological confirmation. Although CT is widely accepted for diagnosing appendiceal perforation, the absence of systematic concordance analysis with intraoperative findings may introduce misclassification bias. Second, the non-perforated comparison group included patients without appendicitis (normal appendix or alternative diagnoses), which may have affected specificity estimates. These patients could have elevated inflammatory markers due to other underlying conditions, potentially underestimating the true diagnostic performance of the evaluated biomarkers for appendicitis specifically. Third, the timing between symptom onset and blood sampling was neither recorded nor standardized. CRP, in particular, is a time-dependent biomarker that increases progressively over 12–24 hours following inflammation. Consequently, early presenters with perforation may have falsely low CRP levels, while late presenters with uncomplicated appendicitis may demonstrate elevated values, potentially affecting diagnostic accuracy.

Fourth, the study population was restricted to patients aged 18–40 years, which, although improving internal validity, limits the external generalizability of the findings to pediatric and elderly populations. Future studies should include a broader age range and consider age-specific diagnostic thresholds. Fifth, this was a single-center study conducted in a tertiary care setting, which may introduce referral and spectrum bias. The relatively high prevalence of perforated appendicitis (40%) likely reflects delayed presentation and selective referral patterns, which may influence predictive values and limit applicability to other clinical settings. Finally, the use of non-probability consecutive sampling and a relatively modest sample size may introduce selection bias and limit the robustness of subgroup analyses.

Conclusion

C-reactive protein, white blood cell count, and serum bilirubin demonstrated significant diagnostic utility in identifying perforated appendicitis, with CRP showing the highest overall diagnostic performance, followed by WBC count and serum bilirubin. CRP exhibited the most favorable balance between sensitivity and specificity, making it a particularly valuable biomarker for clinical risk stratification. In resource-limited settings where access to advanced imaging modalities such as CT is restricted or delayed, these readily available and cost-effective biomarkers can serve as important triage tools to identify patients at higher risk of perforation, thereby facilitating timely referral, imaging prioritization, and early surgical intervention.

Authors' Contributions:

All authors meet the ICMJE criteria for authorship.

Author 1: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

Author 2: Substantial contribution to conception and design, data acquisition, drafting the manuscript, final approval, agreed to be accountable for all the work.

Author 3: Analysis and interpretation of data, drafting the manuscript, final approval, agreed to be accountable for all the work.

Author 4: Analysis and interpretation of data, critical revision, final approval, agreed to be accountable for all the work.

Author 5: Substantial contribution to conception and design, drafting the manuscript, final approval, agreed to be accountable for all the work.

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