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ORIGINAL ARTICLE

# HYPERBILIRUBINEMIA AS A BIOMARKER FOR COMPLICATED APPENDICITIS: A RETROSPECTIVE STUDY

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## Abstract

Introduction: Hyperbilirubinemia has been proposed as a potential biomarker for predicting complicated appendicitis. This study aims to evaluate the role of serum bilirubin in differentiating complicated from uncomplicated appendicitis and to compare its diagnostic performance with other inflammatory markers.

Material and Method: A retrospective analysis was conducted on 30 patients diagnosed with acute appendicitis, including 10 with complicated and 20 with uncomplicated appendicitis. Laboratory parameters, including white blood cell (WBC) count, neutrophil-to-lymphocyte ratio (NLR), platelet (PLT) count, interleukin-6 (IL-6) and serum bilirubin levels, were compared between the two groups. Statistical analyses were performed to determine the predictive value of hyperbilirubinemia for complicated appendicitis.

Results: Patients with complicated appendicitis exhibited significantly higher serum bilirubin levels than those with uncomplicated appendicitis ($p<0.01$). Additionally, CRP and IL-6 were notably elevated in the complicated appendicitis group. However, the combination of serum bilirubin with other inflammatory markers, such as CRP and IL-6, enhanced diagnostic accuracy.

Conclusion: Hyperbilirubinemia is a valuable biomarker for predicting complicated appendicitis and may serve as a useful adjunct to existing diagnostic tools. Further large-scale prospective studies are warranted to validate these findings and establish standardized cutoff values for clinical application.

***Key Words:*** *biomarker; complicated appendicitis; serum bilirubin.*

## Introduction

Acute appendicitis is one of the most common causes of acute abdomen requiring emergency surgical intervention. It is estimated that approximately 7-8% of the population will develop appendicitis during their lifetime, with the highest incidence occurring in adolescents and young adults (1). Despite significant advancements in diagnostic and therapeutic approaches, acute appendicitis remains a challenge due to its variable clinical presentation and potential for rapid progression to complications. From a pathophysiological perspective, appendicitis can be classified into two main categories: 1. uncomplicated appendicitis, characterized by localized inflammation without perforation or gangrene, usually having a favorable outcome with timely intervention; and 2. complicated appendicitis, which involves gangrene, perforation, or abscess formation and is associated with higher morbidity, prolonged hospital stay and an increased risk of postoperative complications. Early differentiation between these forms is essential for determining treatment and preventing complications. Although clinical scoring systems (e.g. Alvarado) and imaging techniques significantly contribute to diagnosis, they are not always sufficiently sensitive or specific in predicting the severity of the inflammatory process (2). Researchers have explored the role of biomarkers such as serum bilirubin, inflammatory cytokines, and hematological indices in distinguishing between uncomplicated and complicated appendicitis.

Hyperbilirubinemia has been proposed as a potential marker for complicated appendicitis, as increased serum bilirubin levels may result from bacterial translocation and endotoxemia, leading to hepatocellular dysfunction and reduced bilirubin clearance (3). Studies have reported that patients with perforated appendicitis exhibit significantly higher bilirubin levels compared to those with uncomplicated cases, suggesting thier utility as a predictor of disease severity (4).

In addition to bilirubin, inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), have been investigated for their role in appendicitis severity. IL-6, a key pro-inflammatory mediator, has shown to correlate with disease progression, with higher levels observed in patients with gangrenous or perforated appendicitis (5). Moreover, hematological indices, such as the neutrophil-to-lymphocyte ratio (NLR) and platelet count (PLT), have gained attention as cost-effective and easily accessible markers. Studies suggest that an elevated NLR is associated with a higher likelihood of complicated appendicitis, reflecting a heightened systemic inflammatory response. Integration of these biomarkers into clinical practice could enhance diagnostic accuracy, especially in borderline cases where imaging findings are inconclusive. While further large-scale prospective studies are needed to validate their predictive value, current evidence suggests that serum bilirubin, IL-6, NLR and other inflammatory markers hold promise in improving early risk stratification for complicated appendicitis (3) (4) (5).

The aim of this study is to evaluate the diagnostic and prognostic potential of serum bilirubin levels in comparison to WBC, NLR, PLT and IL-6 for preoperative identification of complicated appendicitis. Confirming its predictive value could contribute to improving diagnostic strategies and the early identification of high-risk patients.

The primary objective of this study is to investigate the diagnostic and prognostic value of serum bilirubin in distinguishing complicated from uncomplicated appendicitis. Secondary objectives of this study are: 1. to compare the mean values of these biomarkers between patients with complicated (n=10) and uncomplicated appendicitis (n=20); 2. to analyze the correlation between serum bilirubin levels and inflammatory markers (WBC, NLR, PLT, IL-6); and 3. to assess the sensitivity and specificity of each biomarker, individually and in combination, for predicting complications.

## Material and Method

This study is designed as a retrospective, observational, monocentric study conducted in the period from January 2023 to January 2024 at the General Hospital Kumanovo. A total of 30 patients hospitalized with a diagnosis of acute appendicitis and undergoing surgical treatment (either open or laparoscopic appendectomy) were included. Patients were divided into two groups based on histopathological findings:

- **complicated appendicitis group (n=10):** patients with gangrenous or perforated appendicitis or the presence of an abscess; and
- **uncomplicated appendicitis group (n=20):** patients with catarrhal or phlegmonous appendicitis without evidence of complications.

Inclusion Criteria:

- patients over 14 years old diagnosed with acute appendicitis,
- diagnosis confirmed through histopathological analysis after appendectomy, and
- availability of biochemical data, including WBC, NLR, PLT, IL-6, CRP and serum bilirubin.

Exclusion Criteria:

- patients with chronic liver diseases, hemolytic disorders, or previously diagnosed with Gilbert's syndrome,
- pregnant women and patients with confirmed viral hepatitis infection; and
- incomplete medical records in the patient’s documentation.

From medical records, demographic data (age, gender) and the following laboratory parameters were collected: WBC (white blood cell count), NLR (neutrophil-to-lymphocyte ratio), PLT (platelet count), IL-6 (interleukin-6), CRP (C-reactive protein) and total serum bilirubin.

## Results

**Table 1.**

| | gender | age | WBC | NLR | PLT | CRP | TBIL | IL-6 | PHA |
|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 20 | 19 | 15.81 | 250 | 105.1 | 12.7 | 69.3 | phleg |
| 2 | F | 47 | 14.7 | 9.9 | 171 | 146.5 | 18.3 | 89.9 | gang |
| 3 | M | 64 | 18.9 | 9.05 | 358 | 132.7 | 53 | 63.6 | gang |
| 4 | M | 42 | 8.8 | 5.92 | 140 | 81.8 | 25 | 5.76 | phleg |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | F | 26 | 21.5 | 9.4 | 343 | 69.2 | 18 | 13.3 | gang |
| 6 | F | 44 | 10.3 | 4.39 | 324 | 102.2 | 9 | 6.51 | phleg |
| 7 | M | 47 | 12.8 | 16.71 | 204 | 11.6 | 14.3 | 26.1 | phleg |
| 8 | F | 48 | 18.7 | 29.5 | 227 | 41.7 | 15 | 59.6 | phleg |
| 9 | F | 34 | 12.2 | 8.67 | 195 | 6.1 | 7 | 8.66 | phleg |
| 10 | F | 26 | 10.8 | 5.87 | 218 | 43.9 | 9.1 | 2.3 | phleg |
| 11 | M | 21 | 11.9 | 2.86 | 314 | 18.1 | 7.5 | 4.36 | phleg |
| 12 | M | 33 | 8.2 | 3.81 | 221 | 19.8 | 16 | 3.4 | phleg |
| 13 | F | 39 | 18.1 | 7.34 | 249 | 0.5 | 9 | 27.2 | phleg |
| 14 | F | 15 | 8.9 | 4.67 | 306 | 27.4 | 26 | 2.05 | phleg |
| 15 | M | 52 | 4.1 | 6.8 | 114 | 47.3 | 11 | 46.2 | phleg |
| 16 | M | 18 | 15.1 | 4.76 | 190 | 0.3 | 8 | 12.3 | phleg |
| 17 | F | 29 | 7.9 | 2.36 | 182 | 0.3 | 25 | 2.1 | phleg |
| 18 | M | 24 | 19.8 | 11 | 216 | 21.1 | 20.5 | 92.9 | gang |
| 19 | F | 16 | 7.4 | 5.45 | 154 | 107 | 26.7 | 8.05 | phleg |
| 20 | F | 52 | 15.6 | 5.82 | 214 | 34.3 | 13 | 40.3 | gang |
| 21 | M | 77 | 19.9 | 23.14 | 254 | 165.4 | 20 | 282 | phleg |
| 22 | M | 24 | 12.8 | 6.63 | 204 | 6.8 | 23 | 18.8 | gang |
| 23 | M | 25 | 4.2 | 3.44 | 188 | 162.3 | 32 | 563 | gang |
| 24 | M | 14 | 21 | 12.6 | 342 | 149.8 | 28 | 48.3 | gang |
| 25 | M | 31 | 13.9 | 5.65 | 253 | 123.5 | 12.7 | 10.7 | gang |
| 26 | M | 23 | 12.3 | 16.29 | 318 | 62.2 | 13 | 25.8 | phleg |
| 27 | M | 37 | 9.5 | 6.58 | 124 | 1.9 | 12 | 37.4 | phleg |
| 28 | M | 28 | 12.1 | 8.33 | 190 | 18.5 | 16.8 | 25.1 | phleg |
| 29 | M | 22 | 26.1 | 11.6 | 334 | 85 | 26.6 | 93.8 | gang |
| 30 | F | 50 | 10.8 | 3.43 | 230 | 14.4 | 6 | 6.23 | phleg |

Table 1 presents the demographic data (age, gender (M – male and F – female)) and the values of the following laboratory parameters (denoted by their respective abbreviations): white blood cell count x10⁹/L (WBC), neutrophil-to-lymphocyte ratio (NLR), platelet count x10⁹/L (PLT), C-reactive protein in mg/L (CRP), total bilirubin in μmol/L (TBIL), and interleukin-6 in pg/mL (IL-6). The pathological finding (PHA) is categorized into two groups: uncomplicated (phleg) and complicated (gang).

**Table 2.**

| | *age* | *WBC* | *NLR* | *PLT* | *CRP* | *TBIL* | *IL-6* |
|---|---|---|---|---|---|---|---|
| *count* | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 | 30.00 |
| *mean* | 34.27 | 13.58 | 8.93 | 234.23 | 60.22 | 17.81 | 56.50 |
| *std* | 15.35 | 5.38 | 6.17 | 68.44 | 54.75 | 9.83 | 109.85 |
| *min* | 14.00 | 4.10 | 2.36 | 114.00 | 0.30 | 6.00 | 2.05 |
| *0.25* | 23.25 | 9.70 | 4.93 | 190.00 | 15.33 | 11.25 | 6.90 |
| *0.50* | 30.00 | 12.55 | 6.72 | 219.50 | 42.80 | 15.50 | 25.45 |
| *0.75* | 46.25 | 18.55 | 10.73 | 293.00 | 104.38 | 24.50 | 56.78 |
| *max* | 77.00 | 26.10 | 29.50 | 358.00 | 165.40 | 53.00 | 563.00 |

Table 2 presents the basic statistical parameters (mean, standard deviation, minimum and maximum values) for age, WBC, NLR, PLT, CRP, TBIL and IL-6 in patients with appendicitis.

**Table 3.**

| | ***age*** | | ***WBC*** | | ***NLR*** | | ***PLT*** | | ***CRP*** | | ***TBIL*** | | ***IL-6*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean | std | mean | std | mean | std | mean | std | mean | std | mean | std | mean | std |
| *gang* | 32.90 | 15.91 | 16.85 | 6.04 | 8.51 | 2.98 | 262.30 | 73.77 | 93.12 | 57.81 | 24.51 | 11.81 | 103.46 | 164.68 |
| *phleg* | 34.95 | 15.44 | 11.94 | 4.30 | 9.13 | 7.34 | 220.20 | 62.84 | 43.78 | 46.24 | 14.46 | 6.78 | 33.02 | 61.89 |

Table 3 compares the mean values and standard deviations (std) for the two patient groups (uncomplicated (phleg) and complicated appendicitis (gang)).

**Table 4.**

| | ***Test Type*** | ***p-value*** |
|---|---|---|
| *age* | Mann-Whitney U test | 0.72 |
| *WBC* | T-test | 0.04 |
| *NLR* | Mann-Whitney U test | 0.39 |
| *PLT* | T-test | 0.14 |
| *CRP* | Mann-Whitney U test | 0.02 |
| *TBIL* | Mann-Whitney U test | 0.01 |
| *IL-6* | Mann-Whitney U test | 0.01 |

Table 4 presents the p-values from the Mann-Whitney U test and T-test to determine the statistical significance of differences between the groups.

**Table 5.**

| | ***age*** | ***WBC*** | ***NLR*** | ***PLT*** | ***CRP*** | ***TBIL*** | ***IL-6*** |
|---|---|---|---|---|---|---|---|
| *age* | 1.00 | 0.06 | 0.32 | -0.12 | 0.19 | 0.05 | 0.16 |
| *WBC* | 0.06 | 1.00 | 0.55 | 0.58 | 0.20 | 0.14 | -0.05 |
| *NLR* | 0.32 | 0.55 | 1.00 | 0.17 | 0.24 | 0.02 | 0.16 |
| *PLT* | -0.12 | 0.58 | 0.17 | 1.00 | 0.28 | 0.25 | -0.07 |
| *CRP* | 0.19 | 0.20 | 0.24 | 0.28 | 1.00 | 0.49 | 0.55 |
| *TBIL* | 0.05 | 0.14 | 0.02 | 0.25 | 0.49 | 1.00 | 0.34 |
| *IL-6* | 0.16 | -0.05 | 0.16 | -0.07 | 0.55 | 0.34 | 1.00 |

Table 5 presents the correlations between different laboratory parameters, showing which markers are the most strongly associated.

**Table 6.**

| | | ***Spearman Correlation*** | ***p-value*** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| *WBC* | NLR | 0.6918 | 0.0000 |
| *WBC* | PLT | 0.5920 | 0.0006 |
| *WBC* | IL-6 | 0.5532 | 0.0015 |
| *NLR* | WBC | 0.6918 | 0.0000 |
| *NLR* | IL-6 | 0.6570 | 0.0001 |
| *PLT* | WBC | 0.5920 | 0.0006 |
| *CRP* | TBIL | 0.4925 | 0.0057 |
| *CRP* | IL-6 | 0.4563 | 0.0113 |
| *TBIL* | CRP | 0.4925 | 0.0057 |
| *IL-6* | WBC | 0.5532 | 0.0015 |
| *IL-6* | NLR | 0.6570 | 0.0001 |
| *IL-6* | CRP | 0.4563 | 0.0113 |

Table 6 shows the Spearman correlation of numerical variables.

## Discussion

This study investigated the diagnostic and prognostic value of serum bilirubin as a potential biomarker for preoperative identification of complicated appendicitis. The results showed that patients with complicated appendicitis (gangrenous/perforated) had significantly higher serum bilirubin levels compared to those with uncomplicated appendicitis. These findings suggest that bilirubin may be a useful indicator for identifying high-risk patients.

According to the analysis, patients with gangrenous appendicitis had higher mean values of WBC, CRP, TBIL, and IL-6 compared to those with phlegmonous appendicitis. The most significant differences were observed in CRP ($p = 0.02$), TBIL ($p = 0.01$), and IL-6 ($p = 0.01$), indicating that these biomarkers play a crucial role in distinguishing complicated appendicitis.

The strong correlation between TBIL and CRP ($\rho=0.49$, $p=0.0057$) supports the hypothesis that increased bilirubin levels are associated with an elevated inflammatory response. This may be explained by the effect of bacterial endotoxemia on bilirubin metabolism, leading to its elevation in patients with severe inflammation.

These findings are consistent with previous research, which has shown that hyperbilirubinemia is an indicator of complicated appendicitis (4) (6) (7). Studies conducted by Gavriilidis et al. (2019) (8) and Burchart et al. (2013) (9), also suggest that elevated bilirubin levels may signal gangrene or perforation of the appendix. However, larger studies are needed to further validate these results (10).

Some limitations of this study include a small patient sample (n=30), which reduces the statistical power of the analysis. Additionally, this is a retrospective study, meaning biases related to data collection are possible. Future research with a larger sample size and a prospective design could confirm these findings.

## Conclusion

The results of this study indicate that serum bilirubin may serve as a useful biochemical marker for the preoperative identification of complicated appendicitis. Its application, in combination with traditional inflammatory parameters (CRP, WBC, IL-6), could improve diagnostic strategies and optimize surgical treatment.

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