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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

# The potential role of interleukin-6, endotoxin, and C-reactive protein as standard biomarkers for acute appendicitis in adults

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**SUMMARY**

**Introduction/Objective** Acute appendicitis (AA) is by far the most frequent urgent condition in abdominal surgery and numerous biomarkers may help the physician to diagnose and even predict the severity of the disease.

The objective of the paper was to determine the accuracy of C-reactive protein (CRP), interleukin-6, and endotoxin level and compare it with the diagnostic value of Alvarado score (AS) in adults surgically treated for AA.

**Methods** Sixty-seven patients were diagnosed with AA using AS. Prior to surgery serum levels of inflammatory biomarkers were determined and together with AS were respectively compared to the results of histopathological analysis of specimens. The patients were divided into three group according to the histopathological assessment.

**Results** The univariate analysis revealed that the increase of CRP level by one unit increases the probability of complicated AA (CoAA) occurrence by 1% (1.00–1.02, $p < 0.05$). ROC curve analysis has revealed that CRP has better capacity to predict suppurative AA (SAAs)/CoAAs than catarrhal AA (CAA), with the cut-off value of 19.45. The increase of AS value by one unit produced 2.98-fold increase of the probability of CoAA occurrence (1.60–5.57, $p < 0.001$), while positive AS value increases the probability of CoAA occurrence 24.67 times (4.94–123.12; $p < 0.001$). ROC curve analysis demonstrated that AS may predict CoAAs better than CAAs/SAAs, with the cut-off value of 8.50.

**Conclusion** AS and CRP should be routinely used combined as powerful tools for the diagnosis and prediction of complicated AA.

**Keywords:** biomarkers; acute appendicitis; adults

## INTRODUCTION

Acute appendicitis (AA) is by far the most frequent urgent condition in abdominal surgery with reported lifetime risk of 8.6% in men and 6.7% in women [1]. If the initial inflammation progresses is left untreated, the appendix becomes gangrenous and perforates, causing peritonitis and abscess formation, ileus sepsis, and eventually death. This so-called "complicated appendicitis" occurs in approximately 16.5% of patients [2]. Open or laparoscopic appendectomy remains the standard treatment for the condition. However, despite its high incidence, accurate preoperative diagnosis of AA is still challenging. The negative appendectomy rate is 20.6% [2], with peaks in certain categories of patients such as women in childbearing age (30–50%) or young children (30–46%) [3, 4]. The diagnosis of AA is still predominantly clinical, with 80% diagnostic accuracy of the initial algorithm consisting of suggestive history, pain at McBurney's point and leukocytosis [5]. The addition of imaging methods such as ultrasound and especially computerized tomography (CT) increases the diagnostic accuracy and decreases negative appendectomy rate to 10% [6]. Nevertheless, some serious drawbacks limit the diagnostic utility of these radiological modalities. These include high cost and radiation of CT and low sensitivity of ultrasound (failure of appendix visualization in up to 55% of cases) [7, 8].

Numerous biomarkers are associated with AA and may help the physician to diagnose and even predict the severity of the disease. Some of the routinely used biomarkers are widely available but have insufficient diagnostic value [9], while some newly introduced with higher accuracy require costly and time-consuming analysis. When solely used, not a single one of them has all the desired features, which include good diagnostic accuracy and relatively cheap, simple, and time-sparing assay. Therefore, the combination of biomarkers or their use as a part of stratification scores such as the Alvarado score (AS) in conjunction with history data and examination results may improve their sensitivity [10, 11], although the reliability of these scores is limited due to the interpretation subjectivity of history data and examination findings [12]. The aim of this

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study was to determine the accuracy of inflammatory biomarkers C-reactive protein (CRP), interleukin-6 (IL-6), and endotoxin and compare it with the diagnostic value of AS in adults surgically treated for AA.

## METHODS

The study, done in accord with standards of the institutional committee on ethics, included 67 patients that underwent surgery for AA during a period of six months, from January to June 2019 at the Emergency Unit, Niš Clinical Center. There were 35 men (52.2%) and 32 women (47.8%), their median age being 38.7 16.5 years (range: 19–80 years). The patients were diagnosed with AA using AS (Table 1) with diagnostic cut-off value of 6 [13]. Histopathological diagnosis of removed appendices was considered definitive. Prior to surgery, their blood samples were taken and serum levels of CRP, IL-6, and endotoxin were determined. The levels of these inflammatory biomarkers and AS were respectively compared to the results of histopathological analysis of specimens. Surgical treatment of the examined patients included open appendectomy. The severity of appendiceal inflammation was categorized according to the histopathological assessment as presented in Table 2. Gangrenous appendicitis and periappendiceal abscess were categorized as complicated AA (CoAA), as opposed to catarrhal (CAA) and suppurative (SAA) inflammation.

**Table 1.** Alvarado score for diagnosing acute appendicitis

| Clinical signs | Alvarado score |
|---|---|
| Moving pain | 1 |
| Loss of appetite | 1 |
| Nausea and vomiting | 1 |
| Tension in the right lower quadrant | 2 |
| Bloomberg's sign | 1 |
| Fever | 1 (> 37.2°C) |
| Leukocytosis (> 10 × 109) | 2 |
| Polymorphonuclear > 75% | 1 |
| Total | 10 |

**Table 2.** The severity of acute appendicitis according to the histopathological assessment

| Severity grade | Histopathology |
|---|---|
| Catarrhal appendicitis | Intraluminal polymorphonuclear neutrophils |
| Suppurative appendicitis | Mucosal infiltration with inflammatory cells |
| Gangrenous appendicitis (CoAA) | Muscular layer infiltration with inflammatory cells |
| Periappendiceal abscess (CoAA) | Periappendiceal infiltration with inflammatory cells |

CoAA – complicated acute appendicitis

### Statistical data processing

The data are presented in the form of an arithmetic mean and a standard deviation, or in the form of absolute and relative numbers. Group comparisons were performed using the Student's t-test or Mann–Whitney U-test. Analysis of variance (ANOVA) was used to compare continuous variables of three independent groups, including subsequent post hoc tests (Tukey method and Tamhane's T2 test). Alternatively, Kruskal–Wallis test was also used. Assessment of the relationship between categorical variables was done using Pearson's $\chi^2$ test. Diagnostic features of the analyzed parameters (sensitivity and specificity, i.e., predictive value) were assessed using receiver operating characteristic curve (ROC) analyses. P-values < 0.05 were considered statistically significant. Statistical analyses were done using SPSS, Version 16.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

According to age, the patients ranged 18–80 years, with no statistically significant difference in sex representation (numerical sex ratio 1.09 in favor of men) (Figure 1). In terms of age and sex distribution, the largest number of patients who were operated on was in the age group 18–29 years, while the least patients were in the age group of 70 years and older (Table 3).

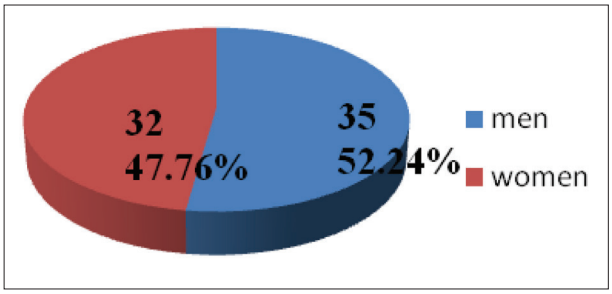


**Figure 1.** Patients' sex distribution

**Table 3.** Distribution of different histopathological categories of acute appendicitis in relation to patients' age and sex

| Age | 18–29y | 30–39y | 40–49y | 50–59y | 60–69y | +70y | Σ |
|---|---|---|---|---|---|---|---|
| Sex | M F | M F | M F | M F | M F | M F | M F |
| CAA (n = 16) | 3 5 | 2 0 | 2 0 | 3 0 | 0 0 | 0 1 | 8 8 |
| SAA (n = 33) | 6 7 | 8 3 | 1 2 | 1 1 | 1 2 | 0 1 | 17 16 |
| CoAA (n = 18 | 2 1 | 4 1 | 0 4 | 0 2 | 4 0 | 0 0 | 10 8 |
| Σ | 11 13 | 12 6 | 3 6 | 4 3 | 5 2 | 0 2 | 35 32 |
| | 24 | 18 | 9 | 7 | 7 | 2 | 67 |

CAA – catarrhal acute appendicitis; SAA – suppurative acute appendicitis; CoAA – complicated acute appendicitis

The distribution of AS values among our patients is presented in Figure 2. Sixty-one patients (91%) had AS values compatible with the diagnosis of AA (6 or greater).

The average value of AS in the examined group of patients was 7.94 ± 1.82, with a median of 8.00, with the lowest value of 2 and the highest 10. CRP values on the total sample ranged 0.6–415.2 mg/L, with an average value of 60.37 ± 79.18 mg/L. In the total sample, the average endotoxin values were 3.42 ± 1.20 MU/mL, with the lowest value of 2.88 MU/mL and the highest of 3.72 MU/mL, with a median of 3.28 MU/mL. IL-6 values ranged 13.17–98.83 pg/mL, with a mean value of 91.40 ± 139.63 pg/mL and a median of 31.33 pg/mL (Table 4).

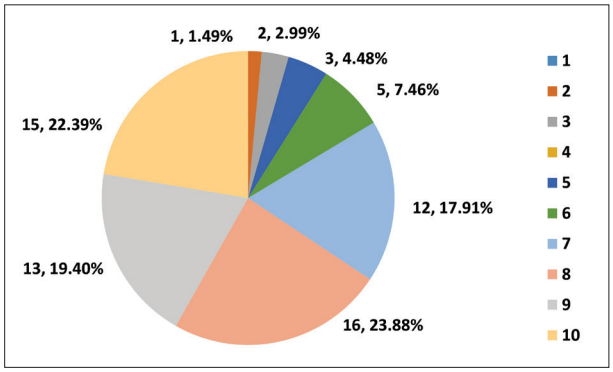


**Figure 2.** Distribution of Alvarado score values among our patients

**Table 4.** Mean values of examined parameters

| Parameter | X ±SD | Me | Min | Max |
|---|---|---|---|---|
| Age (years) | 38.72 ± 16.46 | 36 | 18 | 80 |
| Alvarado score | 7.94 ± 1.82 | 8 | 2 | 10 |
| Endotoxin (MU/mL) | 3.42 ± 1.2 | 3.28 | 2.88 | 3.72 |
| IL-6 (pg/mL) | 91.40 ± 139.63 | 31.33 | 13.17 | 98.83 |
| CRP (mg/L) | 60.37 ± 79.18 | 29.7 | 0.6 | 415.2 |

X – mean value; SD – standard deviation; Me – median; Min – minimum; Max – maximum

**Table 5.** Mean values of examined parameters in relation to Alvarado score values

| Parameter | Alvarado score negative (5 and less) | Alvarado score positive (6 and more) | p |
|---|---|---|---|
| Age (years) | 35.95 ± 16.14 (33) | **42.57 ± 16.42 (39)** | 0.0580 |
| Endotoxin (MU/mL) | 3.49 ± 1.26 (3.32) | 3.32 ± 1.12 (3.17) | 0.7029 |
| IL-6 (pg/mL) | 37 ± 65.62 (16.5) | **167.16 ± 177.12 (84.83)** | 0.0000*** |
| CRP (mg/L) | 42.94 ± 64.46 (18.2) | **84.65 ± 91.79 (52.05)** | 0.0054** |

X – mean value; SD – standard deviation; Me – median;
*p < 0.05;
**p < 0.01;
***p < 0.001 (Student's t-test or Mann–Whitney U-test)

**Table 6.** Mean values of examined parameters in relation to histopathological findings

| Parameter | CAA (n = 16) | SAA (n = 33) | CoAA (n = 18) | p |
|---|---|---|---|---|
| Age (years) | 35.00 ± 17.91 (29) | 36.94 ± 15.94 (35) | **45.28 ± 15.12$^{ab*}$ (46)** | 0.0570 |
| Alvarado score | 6.94 ± 1.18 (7) | 7.7 ± 2.05$^{a*}$ | **9.28 ± 0.83$^{a***b***}$ (9)** | 0.0000*** |
| Endotoxin (MU/mL) | 3.1 ± 0.68 (3.09) | **3.8 ± 1.48$^{ac*}$ (3.39)** | 3 ± 0.68 (3.11) | 0.0409* |
| IL-6 (pg/ml) | 43.73 ± 90.65 (15.41) | 50.6 ± 70.68$^{a**}$ (19.9) | **208.56 ± 197.68$^{ab***}$ (124.58)** | **0.0000*** ** |
| CRP (mg/L) | 19.51 ± 27.77 (15.35) | 56.35 ± 70.68$^{a**}$ (29.9) | **104.05 ± 103.11$^{a***b*}$ (70.15)** | **0.0002*** ** |

X – mean value; SD – standard deviation; Me – median; CAA – catarrhal acute appendicitis; SAA – suppurative acute appendicitis; CoAA – complicated acute appendicitis;
Parameters are given as X ± SD and Me;
[a]*vs* CAA
[b]*vs*. SAA;
[c]*vs*. CoAA;
*p < 0.05;
**p < 0.01;
***p < 0.001 (ANOVA, Kruskal–Wallis test, Student's t-test, Mann–Whitney U-test)

AS positive (6 and more) and histopathological (HP) finding were used as the two most authoritative measures in the final diagnosis of AA. Table 5 shows the basic descriptive indicators of the examined continuous variables for AS negative (5 and less) and AS positive. In the group of patients with AS positive, statistically significantly higher values of IL-6 (p < 0.001) and CRP (p < 0.01) were found.

The basic descriptive indicators of the examined continuous variables in relation to the HP finding of AA are given in Table 6. Statistically significant differences were found between the examined groups of parameters – AS, IL-6, CRP (p < 0.001) and for endotoxin (p < 0.05). The value of AS was statistically significantly higher in CoAA in relation to CAA (p < 0.001) and SAA (p < 0.01), and it was statistically higher in SAA in relation to CAA (p < 0.05). IL-6 in CoAA was statistically significantly higher compared to SAA and CAA alone (p < 0.001). CRP was statistically significantly higher in CoAA compared to CAA (p < 0.001), but also SAA (p < 0.05), while the value in SAA was statistically significantly higher compared to CAA (p < 0.01). Endotoxin values were higher in SAA, compared to CAA, but also in CoAA (p < 0.05). By comparing the values of parameters between the groups, it was determined that the subjects with CoAA were statistically significantly older than those with CAA, as well as those with SAA (p < 0.05).

Table 7 shows the findings of the incidence of elevated values of examined parameters in relation to AS. In the group of patients with AS positive, there was a statistically significantly higher presence of HP findings of CoAA (p < 0.001) and IL-6 (p < 0.01). No patient with AS positive had IL-6 values < 5.9 pg/mL.

Statistically significant different representation of findings compared to HP finding of AA was found for IL-6, CRP (p < 0.01) and endotoxin (p < 0.01). The prevalence of AS, IL-6, and CRP findings above the reference values is the highest in CoAA and the lowest in CAA, while the finding of endotoxin above the reference values is most prevalent in SAA. By comparing the values of the examined parameters between the groups with HP findings of AA, it was found that the findings of AS positive were statistically more prevalent in CoAA compared to SAA and catarrhal findings separately (p < 0.001) (Table 8).

Univariate logistic regression analysis for modelling event probabilities was applied in order to assess whether examined parameters may predict the severity of appendiceal inflammation definitively determined by histopathological analysis (Table 9). Positive correlation was found for AS and CRP: an increase of CRP value by one unit increases the probability of CoAA occurrence by 1% (1.00–1.02, p < 0.05); an increase of AS value by one unit produced 2.98-fold increase of the probability of CoAA occurrence (1.60–5.57, p < 0.001), while positive AS value increases the probability of CoAA occurrence 24.67 times (4.94–123.12; p < 0.001). Diagnostic potential (sensitivity and specificity) of these two parameters (CRP and AS) was assessed using ROC curve analysis and two cut-off values were determined: one for the distinction between CAAs

**Table 7.** The incidence of elevated values of examined parameters in relation to Alvarado score

| Parameter | Value | Alvarado score (%) | | p |
|---|---|---|---|---|
| | | negative | positive | |
| Endotoxin | normal<br>elevated | 64.1<br>35.9 | 75<br>25 | 0.3466 |
| IL-6 | normal<br>elevated | 30.77<br>69.23 | 0<br>**100** | **0.0013**** |
| CRP | normal<br>elevated | 17.95<br>82.05 | 14.29<br>85.71 | 0.7506 |
| Histopathology | CAA and SAA<br>CoAA | 94.87<br>5.13 | 42.86<br>**57.14** | 0.0000*** |

CAA – catarrhal acute appendicitis; SAA – suppurative acute appendicitis; CoAA – complicated acute appendicitis;
**p < 0.01;
***p < 0.001 ($\chi^2$ test)

**Table 8.** The incidence of elevated values of examined parameters in relation to histopathological findings

| Parameter | Value | Histopathology (%) | | | p |
|---|---|---|---|---|---|
| | | CAA | SAA | CoAA | |
| Alvarado score | normal<br>elevated | 93.75<br>6.25 | 66.67<br>33.33 | 11.11<br>**88.89ab***** | **0.0000***** |
| Endotoxin | normal<br>elevated | 81.25<br>18.75 | 51.52<br>**48.48a*c***** | 88.89<br>11.11 | **0.0105*** |
| IL-6 | normal<br>elevated | 43.75<br>56.25 | 15.15<br>84.85 | 0<br>**100a**** | **0.0050**** |
| CRP | normal<br>elevated | 43.75<br>56.25 | 12.12<br>87.88a* | 0<br>**100a**** | **0.0018**** |

CAA – catarrhal acute appendicitis; SAA – suppurative acute appendicitis; CoAA – complicated acute appendicitis;
[a]*vs.* CAA;
[b]*vs.* SAA;
[c]*vs.* CoAA;
*p < 0.05;
**p < 0.01;
***p < 0.001 ($\chi^2$ test)

**Table 9.** Results of univariate logistic regression analysis assessing the probability of AA histopathology prediction by examined parameters

| Parameter | OR | Limits 95% CI | | p |
|---|---|---|---|---|
| | | Lower | Upper | |
| AS | 2.98 | 1.60 | 5.57 | **0.0006***** |
| Positive AS value | 24.67 | 4.94 | 123.12 | **0.0001***** |
| CRP | 1.01 | 1 | 1.02 | **0.0165*** |
| Elevated CRP | - | 0 | - | 0.9987 |

AS – Alvarado score; OR – odds ratio (between catarrhal acute appendicitis and suppurative acute appendicitis on one side and complicated acute appendicitis on the other); CI – confidence interval;
*p < 0.05;
**p < 0.01;
***p < 0.001

and SAAs/CoAAs, and the other for the distinction between CAAs/SAAs and CoAAs. Based on the values of the parameters, it is evident that in this case, slightly better

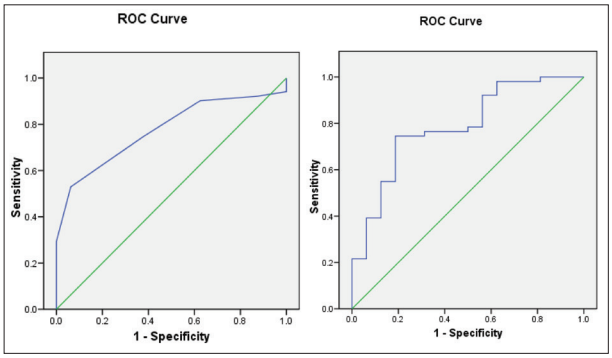


**Figure 3.** Receiver operating characteristic (ROC) curve analysis presenting predictive features of a) Alvarado score and b) CRP for distinction between catarrhal acute appendicitis and suppurative acute appendicitis / complicated acute appendicitis

**Table 10.** Receiver operating characteristic curve coordinates presenting predictive features of Alvarado score (AS) and C-reactive protein (CRP) for distinction between catarrhal acute appendicitis and suppurative acute appendicitis / complicated acute appendicitis

| AS | Se | Sp | Se + Sp | CRP | Se | Sp | Se + Sp |
|---|---|---|---|---|---|---|---|
| 4 | 0.941 | 0.000 | 0.941 | 16.3 | 0.765 | 0.688 | 1.452 |
| 5.5 | 0.922 | 0.125 | 1.047 | 17.3 | 0.745 | 0.688 | 1.433 |
| 6.5 | 0.902 | 0.375 | 1.277 | 18.6 | 0.745 | 0.750 | 1.495 |
| 7.5 | 0.745 | 0.625 | 1.370 | **19.45** | **0.745** | **0.813** | **1.558** |
| **8.5** | **0.529** | **0.938** | **1.467** | 20.75 | 0.725 | 0.813 | 1.538 |
| 9.5 | 0.294 | 1.000 | 1.294 | 22.15 | 0.706 | 0.813 | 1.518 |
| 11 | 0.000 | 1.000 | 1.000 | 23.7 | 0.686 | 0.813 | 1.499 |

Se – sensitivity; Sp – specificity

diagnostic characteristics are shown by CRP in comparison to AS. The area under the curve is 0.787, with a standard estimation error of 0.065, with a statistical significance of p = 0.0006 (p < 0.001). The cut-off value is 19.45. Although it has a slightly wider confidence interval (0.659–0.914) compared to AS, it has significantly more sensitivity (74.51), with slightly less specificity and greater overall accuracy (Figure 3, Tables 10 and 11).

On the other hand, it was demonstrated that AS may predict CoAAs better than CAAs/SAAs. The area under the curve is 0.823 with a standard estimation error of 0.053, with a statistical significance of p = 0.0001 (p < 0.001). The cut-off value is 8.50. It has a relatively narrow confidence interval (0.719–0.927), the best ratio of sensitivity and specificity (88.89% and 75.51%, respectively), the highest values of positive predictive value and negative predictive value and overall accuracy, with slightly lower specificity and higher overall accuracy (Figure 4, Tables 12 and 13).

**Table 11.** Diagnostic features of Alvarado score (AS) and CRP for distinction between catarrhal acute appendicitis and suppurative acute appendicitis / complicated acute appendicitis

| Parameter | Area below ROC curve (95% CI) | SE | p | Cut-off | Se (%) | Sp (%) | PPV (%) | NPV (%) | OA (%) |
|---|---|---|---|---|---|---|---|---|---|
| AS | 0.775 (0.662–0.889) | 0.053 | 0.0001*** | 8.5 | 52.94 | 93.75 | 96.43 | 37.5 | 62.69 |
| CRP | 0.787 (0.659–0.914) | 0.065 | 0.0006*** | 19.45 | 74.51 | 81.25 | 92.68 | 44.83 | 76.12 |

ROC – receiver operating characteristic; CI – confidence interval; SE – standard error; Se – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value; OA – overall accuracy; CRP – C-reactive protein
*p < 0.05;
***p < 0.001

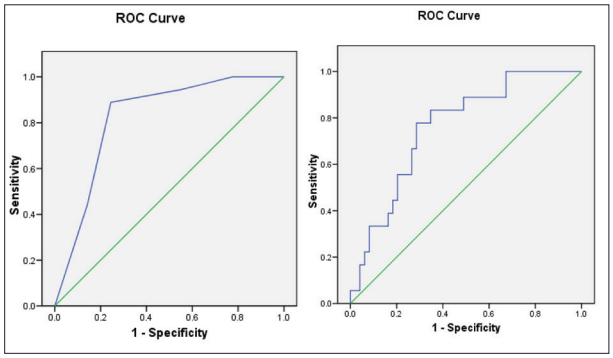


**Figure 4.** Receiver operating characteristic (ROC) curve analysis presenting predictive features of a) Alvarado score and b) C-reactive protein for distinction between complicated acute appendicitis and catarrhal acute appendicitis / suppurative acute appendicitis

**Table 12.** Receiver operating characteristic curve coordinates presenting predictive features of Alvarado score (AS) and C-reactive protein (CRP) for distinction between complicated acute appendicitis and catarrhal acute appendicitis / suppurative acute appendicitis

| AS | Se | Sp | Se + Sp | CRP | Se | Sp | Se + Sp |
|---|---|---|---|---|---|---|---|
| 4 | 1.000 | 0.061 | 1.061 | 32.35 | 0.778 | 0.653 | 1.431 |
| 5.5 | 1.000 | 0.122 | 1.122 | 33.95 | 0.778 | 0.673 | 1.451 |
| 6.5 | 1.000 | 0.224 | 1.224 | 35.55 | 0.778 | 0.694 | 1.472 |
| 7.5 | 0.944 | 0.449 | 1.393 | **40.4** | **0.778** | **0.714** | **1.492** |
| **8.5** | **0.89** | **0.760** | **1.644** | 45.25 | 0.667 | 0.714 | 1.381 |
| 9.5 | 0.444 | 0.857 | 1.302 | 47.9 | 0.667 | 0.735 | 1.401 |
| 11 | 0.000 | 1.000 | 1.000 | 49.5 | 0.611 | 0.735 | 1.346 |

Se – sensitivity; Sp – specificity

## DISCUSSION

Despite the constant high frequency of AA, its timely and accurate diagnosis may still be elusive. A wide variety of biomarkers has been shown associated with AA and potentially able to reduce the risk of misdiagnosed inflammation and/or negative appendectomy. While traditional markers such as leukocytes are cheap and have relatively poor diagnostic accuracy, some of the novel ones such as IL-6 have been shown to have a higher predictive value, but are more expensive and time-consuming. Thus, the quest for the ideal biomarker to be used solely or combined with other parameters or as a part of stratification scores has been in focus for quite a while now.

IL-6 is a proinflammatory cytokine, mediator of acute phase reaction, and is secreted during inflammatory process and neutrophil recruitment following the invasion of bacteria to the appendix [14, 15]. Some of the previous studies have shown its relatively high sensitivity (73–84%) and low specificity (46–72%) for diagnosing AA and even higher sensitivity (up to 91%) and lower specificity (37%) for diagnosing perforated appendicitis [16, 17]. Elevated serum IL-6 levels were found in the majority of our patients (55, 82.09%, $p < 0.001$). In relation to AS, in our study serum IL-6 levels were significantly both higher ($p < 0.001$) and more frequently elevated ($p < 0.01$) in patients with positive AS values as compared to ones with negative AS (Tables 5 and 7, respectively). Also, in relation to histopathology, IL-6 levels were significantly both higher ($p < 0.001$) and more frequently elevated ($p < 0.01$) in patients with CoAA in comparison to the ones with CAA/SAA (Tables 6 and 8, respectively). However, univariate logistic regression analysis failed to demonstrate the predictive capacity of IL-6 for the severity of appendiceal inflammation. These results are consistent with available literature data reporting good overall performance of IL-6 in terms of sensitivity, but still not specific enough especially for diagnosing CoAA and associated with higher cost and time consuming [18].

CRP is synthesized in the liver as an acute-phase reactant to infection or inflammation. Its serum levels rapidly increase within the first 12h from the onset of symptoms, which is followed by an equally fast normalization. CRP is reported as a useful tool for the diagnosis of AA with its high serum levels indicating suppurative and gangrenous evolution of the inflammation or appendiceal perforation. Multiple studies have demonstrated its high sensitivity (93.6–96.6%) [19–21]. However, it reportedly lacks specificity and cannot be used to distinguish between sites of infection [22]. Elevated serum CRP levels were also found in the majority of our patients (56, 83.58%, $p < 0.001$). In relation to AS, in our study, serum CRP levels were significantly higher ($p < 0.01$) in patients with positive AS values as compared to ones with negative AS (Table 5). However, in contrast to IL-6, although elevated CRP levels were more frequent in patients with positive AS than in those with negative AS, this difference lacks statistical significance (Table 7). In relation to histopathology, CRP levels were significantly both higher ($p < 0.001$) and more frequently elevated ($p < 0.01$) in patients with CoAA in comparison to those with CAA/SAA (Tables 6 and 8, respectively). Also, as opposed to IL-6, univariate logistic regression analysis has demonstrated the capacity of CRP to predict the severity of appendiceal inflammation: it was shown that the increase of CRP level by one unit increases the probability of CoAA occurrence by 1% (1.00–1.02, $p < 0.05$) (Table 9). Furthermore, ROC curve analysis has revealed that CRP has better capacity to predict SAAs/CoAAs than CAA, with the cut-of value of 19.45 (Figure 3, Tables 10

**Table 13.** Diagnostic features of Alvarado score (AS) and C-reactive protein (CRP) for distinction between complicated acute appendicitis and catarrhal acute appendicitis / suppurative acute appendicitis

| Parameter | Area below ROC curve (95% CI) | SE | p | Cut-off | Se (%) | Sp (%) | PPV (%) | NPV (%) | OA (%) |
|---|---|---|---|---|---|---|---|---|---|
| AS | **0.823 (0.719–0.927)** | **0.053** | **0.0001**** | **8.5** | **88.89** | **75.51** | **57.14** | 72.55 | **79.1** |
| CRP | 0.789 (0.638–0.879) | 0.062 | 0.0013** | 40.4 | 77.78 | 71.43 | 50 | 66.04 | 73.13 |

ROC – receiver operating characteristic; CI – confidence interval; SE – standard error; Se – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value; OA – overall accuracy;
*$p < 0.05$;
**$p < 0.01$

and 11). These results clearly demonstrate that CRP levels contribute the precise AA diagnosis, the prediction of the severity of inflammation, and may serve as independent markers for CoAAs. Nevertheless, as not specific for AA, its interpretation during the decision-making process should be combined with the analysis of additional diagnostic parameters.

Since AA is a bacterial infection, it may be expected that the severity of inflammation is dependent on the amount of a range of extracellular products and cell-wall constituents produced and released by bacteria. These products stimulate the local and systemic inflammatory response eventually leading to the sepsis and shock. Among these products, endotoxin (lipopolysaccharide complex from the outer membrane of Gram-negative bacteria such as *Escherichia coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus influenzae*, *Bordetella pertussis* and *Vibrio cholera*) is one of the most important ones. During an infectious disease, endotoxins released from bacterial cells significantly contribute to the disease pathophysiology and symptoms' development. However, elevated serum endotoxin levels were found in only 21 (31.34%) of our patients. In our study, serum endotoxin levels did not corelate to AS values, i.e., were not significantly neither higher nor more frequently elevated in patients with positive AS values as compared to those with negative AS (Tables 5 and 7, respectively). In relation to histopathology, endotoxin levels were significantly both higher ($p < 0.05$) and more frequently elevated ($p < 0.05$) only in patients with SAA in comparison to the ones with both CAA and CoAA (Tables 6 and 8, respectively). Univariate logistic regression analysis failed to demonstrate the predictive capacity of endotoxin for the severity of appendiceal inflammation. These results of our study indicate a rather modest pathogenic activity of endotoxins and, hence, their smaller diagnostic value. In comparison to bacterial exotoxins, endotoxins are less potent, less specific in their action, and remain stable within the cell membrane until its disintegration during the first hours of bacterial infection. This may explain their relatively low serum levels in patients with CAAs. Also, endotoxins stimulate natural immunity and proinflammatory activity (production of cytokines, activation of the complement and coagulation cascades) [23], thus preventing their high levels in patients with CoAA.

AS enables risk stratification in patients presenting with abdominal pain suspected of AA [13]. However, although AS is often sufficient when probability of AA is intermediate and physician is in doubt, further investigations (ultrasound, CT) or additional biomarkers determination are recommended [24]. In our study, 61 patients (91%) had AS values compatible with the diagnosis of AA (6 or greater). In relation to histopathology, AS values were significantly both higher ($p < 0.001$) and more frequently elevated ($p < 0.001$) in patients with CoAA in comparison to those with CAA/SAA (Tables 6 and 8, respectively). On univariate logistic regression analysis it was shown that an increase of AS value by one unit produced 2.98-fold increase of the probability of CoAA occurrence (1.60–5.57, $p < 0.001$), while positive AS value increases the probability of CoAA occurrence 24.67 times (4.94–123.12; $p < 0.001$) (Table 9). On ROC curve analysis, it was demonstrated that AS may better predict CoAAs than CAAs/SAAs, with the cut-off value of 8.50 (Figure 4, Tables 12 and 13). These data illustrate very good predictive capacity of AS, especially for determining the possibility of CoAA. This is consistent with the results of other researchers reporting AS as a supreme diagnostic aid [25].

## CONCLUSION

The present study has demonstrated excellent and complementary diagnostic features of both AS and CRP, especially their capacity for predicting complicated forms of AA. Despite good sensitivity and overall performance, IL-6 was not shown useful due to the lack of specificity for diagnosing CoAA, higher cost, and its time consumption. Endotoxin levels were not significantly elevated in our patients and showed rather modest pathogenic activity and, hence, an insignificant diagnostic value. AS and CRP should be routinely used combined as powerful tools for diagnosing and predicting complicated AA.

**Conflict of interest:** None declared.

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## Потенцијална улога интерлеукина-6, ендотоксина и Ц-реактивног протеина као стандардних биомаркера акутног апендицитиса код одраслих

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**САЖЕТАК**

**Увод/Циљ** Акутни апендицитис (АА) најчешће је ургентно стање у абдоминалној хирургији, а бројни биомаркери могу помоћи лекару да дијагностикује, чак и предвиди тежину болести.

Циљ рада је био да се утврди тачност Ц-реактивног протеина (ЦРП), интерлеукина-6 (*IL*-6) и ендотоксина и упореди са дијагностичком вредношћу Алварадо скора (АС) код одраслих болесника хируршки третираних због АА.

**Методе** Код 67 болесника дијагностикован је АА коришћењем АС. Пре операције одређени су нивои инфламаторних биомаркера у серуму и заједно са АС су поређени са резултатима хистопатолошке анализе узорака. Болесници су према хистопатолошком налазу подељени у три групе.

**Резултати** Униваријантна анализа открила је да повећање нивоа ЦРП за једну јединицу повећава вероватноћу јављања компликованог АА (*CoAA*) за 1% (1,00 до 1,02, $p < 0,05$). Анализа *ROC* кривуље открила је да ЦРП има бољи капацитет за предвиђање супуративних АА (*SAA*)/*CoAAs* у односу на катаралне АА (*CAA*), са *cut-off* вредношћу од 19,45. Повећање вредности АС за једну јединицу довело је до 2,98 пута веће вероватноће појаве *CoAA* (1,60 до 5,57, $p < 0,001$), док позитивна вредност АС (6 и више) повећава вероватноћу појаве *CoAA* 24,67 пута (4,94 до 123,12; $p < 0,001$). Анализа *ROC* кривуље је показала да АС може боље предвидети *CoAAs* него *SAAs/CAAs*, са *cut-off* вредношћу 8,50.

**Закључак** АС и ЦРП треба рутински користити у комбинацији, као снажне параметре за дијагнозу и предвиђање компликованих АА.

**Кључне речи:** биомаркери; акутни апендицитис; одрасли