HEALTH SCIENCES

Diagnostic Value of Serum Cytokines in Predicting a Complicated Acute Appendicitis

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Abstract: The diagnostic role of serum cytokines depends on the etiology and pathogenesis of acute appendicitis but the clinical significance of these cytokines in the differential diagnosis of complicated acute appendicitis remains unclear. To investigate the prediction of progression and diagnostic values of interleukin-6, interleukin-1 beta, and tumor necrosis factor-alpha in complicated acute appendicitis. This study was conducted in 100 patients with a definitive diagnosis of acute appendicitis and 20 individuals assigned for the control group. Venous blood was collected to assess biochemical tests, as well as interleukin-6, interleukin-1β, and tumor necrosis factor-α levels. Serum levels of all parameters were dramatically higher in the complicated group compared with uncomplicated. Duration of hospitalization, rates of postoperative infection, intraabdominal abscess, and re-hospitalization were higher in complicated group. Cut-off points of WBC, CRP, NLR, interleukin-6, interleukin-1β and tumor necrosis factor-α were $13.5 \times 10^3/\mu L$, 1.92 mg/dL, 6.09, 23.4 pg/mL, 5.6 pg/mL and 24 pg/mL (p=0.0014, p<0.001, p=0.009, respectively and p<0.001 for the rest). AUC of interleukin-6 was larger than AUCs of all other parameters, suggesting the highest predicting power of interleukin-6 among other parameters. Serum interleukin-6, interleukin-1β, and tumor necrosis factor-α levels are valuable diagnostic parameters to predict a complicated acute appendicitis.

Key words: Acute Appendicitis, interleukin-6, interleukin-1β, ROC analysis, TNF-α.

INTRODUCTION

Appendicitis is one of the most common diseases which necessitates an emergency intervention by surgery (Rubér 2012). It is usually managed by the appendectomy with low morbidity and mortality. Complications such as perforation with abscess formation and localized or four-quadrant peritonitis occur in about 15% of patients (Drake et al. 2014). There are several indications for the diagnosis of acute appendicitis (AA) which is divided into two types as complicated (advanced/perforated) and uncomplicated (phlegmonous/non-perforated) appendicitis. Complicated AA progresses to gangrene and perforation while uncomplicated

one resolves spontaneously (Rubér 2012). In cases of a perforated appendix, the abdominal pain intensifies and is often of a more diffuse character, with a possible development of rigidity, tachycardia and elevation of temperature above 39°C. The pain may occasionally improve somewhat after rupture of the appendix because of relief of visceral distension, but it does not disappear (Rubér 2012). The diagnosis can be supported by adding laboratory investigations. Inflammatory variables temperature, white blood cell (WBC) counts, C-reactive protein (CRP), neutrophil/lymphocyte ratio (NLR) have been shown to be as crucial as clinical findings (direct and rebound abdominal tenderness and

guarding), especially in cases with complicated AA (Andersson et al. 2000). However, these indications are not specific to any inflammatory disease, and in many of them, the blood WBC and CRP levels are found to be high. Today, although it is used in the diagnosis of AA, sensitivity and specificity are low without the support of physical examination and imaging techniques (Bolandparvaz et al. 2004).

The diagnostic role of some inflammatory parameters is based on the etiology and pathogenesis of AA in which a local edema secondary to the impaired blood and lymphatic flow is observed (Turkyilmaz et al. 2006, Lamps 2008). After the appendicular epithelium fails to maintain its bacterial barrier, a massive bacterial invasion into the submucosal layers occurs (Lamps 2008), resulting in the activation of immune system including the local infiltration by T cells, monocytes, and natural killer cells. Locally interleukins and chemokines are released to recruit these cells (Arlt et al. 2015). Cytokines are biological active substance of polypeptides and glycoproteins which participate in the cellular immunity in response to specific inflammatory process in the body (Dalal et al. 2005). Several previous studies examining these immunologic responses to bacterial infection within the intestinal lumen have shown specific cytokine production which help in the diagnosis of AA (Arlt et al. 2015, Rivera-Chavez et al. 2003, Zviedre et al. 2016). In the plasma of patients with uncomplicated local infection/inflammation, high concentrations of several anti-inflammatory cytokines [interleukin (IL)-4, IL-6, IL-10, IL-1Ra], while the classical proinflammatory ones tumor necrosis factor-alpha (TNF-α), IL-1β, interferon-gamma (IFN-γ), IL-12) were found in low concentrations (Rivera-Chavez et al. 2003). These findings were accentuated in patients with more severe local inflammation. The aim of this prospective single-center study was to determine the diagnostic capacity of serum IL-6, IL-1β and TNF-α levels in the patients with complicated or uncomplicated AA. Additionally, the diagnostic accuracy of these cytokines as a predictor of the severity of appendicitis was assessed by comparing with the diagnostic features of WBC, NLR and CRP.

MATERIALS AND METHODS

Patients

A hundred patients diagnosed with an acute appendicitis, with an American Society of Anesthesiologists (ASA) score of I–III, who underwent a laparoscopic appendectomy in our general surgery clinic between November 2019 and January 2020 were selected for this prospective study. The control group consisted of 20 adults (above 16 age), which were selected from the admitted volunteers to our outpatient clinics for routine control were included to study. The control group consists of healthy volunteers who do not have autoimmune, chronic, or other acute illnesses that could change baseline levels of evaluated mediators. The patients without an appendicitis diagnosis, deficiencies in the tests, histopathologically confirmed as negative cases of the appendectomy, those who did not agree to have surgery, subjects who were alcoholic, smokers, had obesity (BMI >30), malignant diseases and systemic inflammatory diseases were excluded from the acute appendicitis group. Moreover, subjects who were pregnant, appendicitis-operated, taking vitamins or antioxidant supplements were also excluded.

A physical examination, routine laboratory tests and abdominal ultrasonography (USG) and computerized tomography (CT) were performed to the patients, who presented with an abdominal pain. The diagnoses were also confirmed by a surgeon intraoperatively according to edema, hyperemic, erectile and inflammated

appendix. Definitive diagnosis was given from extracted specimen following operation by pathologist for all patients. In some cases with complicated appendicitis, pathological specimen did not obtain because of the abscess formation from the common gangrenous and necrosis in the appendix tissue.

.To determine the sample size of the groups and to conduct power analysis for the predictive value of IL-6, GPower 3.1.9.4 program was used. The effect size for IL-6 value was determined as 0.78 calculated from the outcomes of pilot application (n=10 for control and n=50 for the patient group). Under the conditions of 0.05 Type I error rate and 90% power, the minimum numbers of cases needed for the study were determined as a total of 120 patients which include 20 for the control group and 100 for acute appendicitis group. IL-6 and the other cytokines were used for distinction between the complicated and uncomplicated appendicitis.

The study was approved by the local ethics committee (2019/451).

Laboratory Tests

Blood samples were taken preoperatively and before any medical treatment. The normal CRP (1 to 50 mg/dL), WBC (4 to $11 \times 10^3/\mu L$) and NLR values were defined according to the reference values used in our hospital laboratories. Blood samples were obtained by venipuncture into ethylenediaminetetraacetic acid (EDTA) blood collection tubes and immediately centrifuged at 2500 rpm for 10 minutes. After centrifugation, plasma samples were stored at -80 ºC until analysis (maximum, 3 months). The samples were thawed only once. Serum IL-6, IL-1β, and TNF-α levels were determined using the Enzyme Linked Immunosorbent Assay (ELISA) method according to the manufacturer's recommendations with the Diaclone, France. IL-6, IL-1β and TNF-α levels reported as pg/mL were measured in blood samples obtained from both the acute appendicitis and control groups.

Surgery

The abdominal cavities of these patients were explored during the laparoscopic appendectomy. Only those without any additional intra-abdominal inflammatory pathologies were included the study. Uncomplicated acute appendicitis was defined as catarrhal and phlegmonous appendicitis while complicated acute appendicitis was defined as acute appendicitis in which perforation, gangrenous, or an intra-abdominal abscess (Al-Omran et al. 2003).

Statistical Analysis

The data were examined for normality of distribution by the Kolmogorov Smirnov test. Data without normal distribution are expressed as median [minimum-maximum]. In case of rejection of normality, nonparametric Mann-Whitney U test was used to compare two independent variables and Kruskal Wallis H test for three group comparisons. Multiple comparisons were performed by Dunn's multiple comparisons test with Bonferonni correction. Categorical variables were analyzed by the Chi-Square test. P values of <0.05, <0.01 and <0.001 were considered as statistically significant.

A receiver operating characteristic (ROC) curve was constructed to assess sensitivity and specificity as well as optimal cut points for each biochemical parameter to diagnose acute appendicitis. Healthy group was used as negative group for ROC analysis. The changes in the related areas under curve (AUC) were tested by using the DeLong test (DeLong et al. 1988). The optimal cut-off values were determined by using Youden's index.

Statistical analyses were performed using NCSS 11 (Number Cruncher Statistical System,

2017 Statistical Software) and MedCalc Statistical Software version 18 (MedCalc Software bvba, Ostend, Belgium). Power analysis was performed by G*Power 3.1.9.2 (Faul et al. 2009).

RESULTS

There were 120 subjects divided into three groups as control group (n=20), uncomplicated acute appendicitis group (n=66) and complicated acute appendicitis group (n=34). Of the patients, 38 (57.6%) were male in uncomplicated group, 24 (70.6%) were male in complicated group. The control group included 9 (45%) females and 11 (55%) males with a mean age of 35.5±12.8 [16-63] years. There was no significant difference for the age and gender among groups (Table I). The patients with uncomplicated acute appendicitis showed significantly higher levels of WBC and NLR than the control group (p<0.01) while patients with complicated acute appendicitis had significantly highest levels of CRP, WBC and NLR compared to the uncomplicated and control groups (p<0.0001, p<0.01, p<0.05; p<0.001, respectively). Additionally, the serum levels of IL-1β and TNF-α in patients with uncomplicated acute appendicitis were considerably higher than the control groups (p<0.001 and <0.05, respectively) while serum levels of IL-6, IL-1β and TNF-α in patients with complicated acute appendicitis were dramatically higher than the the uncomplicated and control groups (p<0.0001, p<0.01, p<0.0001; p<0.001, respectively) (Figure 1) (Table I).

Expectedly, in the complicated acute appendicitis group, USG showed significantly larger diameter of the appendix (p=0.011), and the majority (67.6%) of patients had a periappendiceal fluid while minority (9.1%) in the uncomplicated group had a fluid (p<0.0001). In

Table I. Characteristics and biochemical parameters of the control group and the patients with uncomplicated or complicated acute appendicitis.

		Control Group (n = 20)	Uncomplicated AA (n=66)	Complicated AA (n=34)	P value
Age (years)	X ± SD [Range]	35.5 ± 12.8 [16-63]	33.9 ± 11.5 [20-72]	35.8 ± 18.8 [16-78]	0.727
Gender N %	Male Female	11 (55) 9 (45)	38 (57.6) 28 (42.4)	24 (70.6) 10 (29.4)	0.380
CRP (mg/dL)	X ± SD [Range]	0.64 ± 0.52 [0.06-1.9]	2.83 ± 4.2 [0.06-19.8]	10.77 ± 10.7*** [0.08-42.88]	**<0.0001**
WBC (x10^3/µL)	X ± SD [Range]	8.73 ± 2.08 [4.9-11.7]	12.40 ± 4.8** [4.9-26.4]	14.76 ± 3.9*** [5.96-21.2]	**<0.01**
NLR	X ± SD [Range]	2.98 ± 1.26 [1.31-5.29]	7.36 ± 8.2** [1.3-48.0]	9.34 ± 6.2*** [1.1-23.5]	**<0.05**
IL-6 (pg/mL)	X ± SD [Range]	10.7 ± 5.6 [4-25]	13.3 ± 7.8 [3.2-36]	98.8 ± 73.9*** [16.2-210]	**<0.0001**
IL-1β (pg/mL)	X ± SD [Range]	3.1 ± 1.3 [1.2-5.2]	7.6 ± 4.9*** [1-25]	11.9 ± 7.8*** [3.7-35]	**<0.01**
TNF-α (pg/mL)	X ± SD [Range]	12.1 ± 5.7 [2-31]	18.36 ± 12.2* [4-76]	36.4 ± 17.7*** [20.3-76]	**<0.0001**

*p<0.05, **p<0.01, ***p<0.001 vs control group. X ± SD: Mean ± Standard deviation, CRP: C-reactive protein, WBC: white blood cell, NLR: Neutrophil/Lymphocyte ratio, IL-6: Interleukin 6, IL-1β: Interleukin 1 beta, TNF-α: Tumor necrosis factor-alpha.

terms of the presence of hypertension, diabetes, respiratory disorders, and of ASA scores, there were no significant difference between the uncomplicated and complicated groups (p>0.05) (Table II).

Table III shows the comparisons of the postoperative parameters of the patients with uncomplicated and complicated acute appendicitis. The time of hospitalization, the rate of postoperative infection, presence of the intraabdominal abscess and rate of re-hospitalization were significantly higher in the complicated group compared with the uncomplicated group (p<0.0001, p<0.001, p<0.01

and p<0.01, respectively). However, there were no patients with postoperative fistula and no patients applied to the hospital for a second time in the uncomplicated group, while 2 patients (5.9%) in the complicated group had a postoperative fistula (p>0.05) and 6 patients were re-hospitalized (p<0.01).

Comparing the diagnostic test results with ROC analysis (Table IV), variables of WBC (AUC=0.678, p=0.0014), CRP (AUC=0.815, p<0.001), NLR (AUC=0.652, p=0.009), IL-6 (AUC=0.968, p<0.001), IL-1β (AUC=0.697, p<0.001) and TNF-α (AUC=0.874, p<0.001) were significant parameters in predicting the presence of complicated
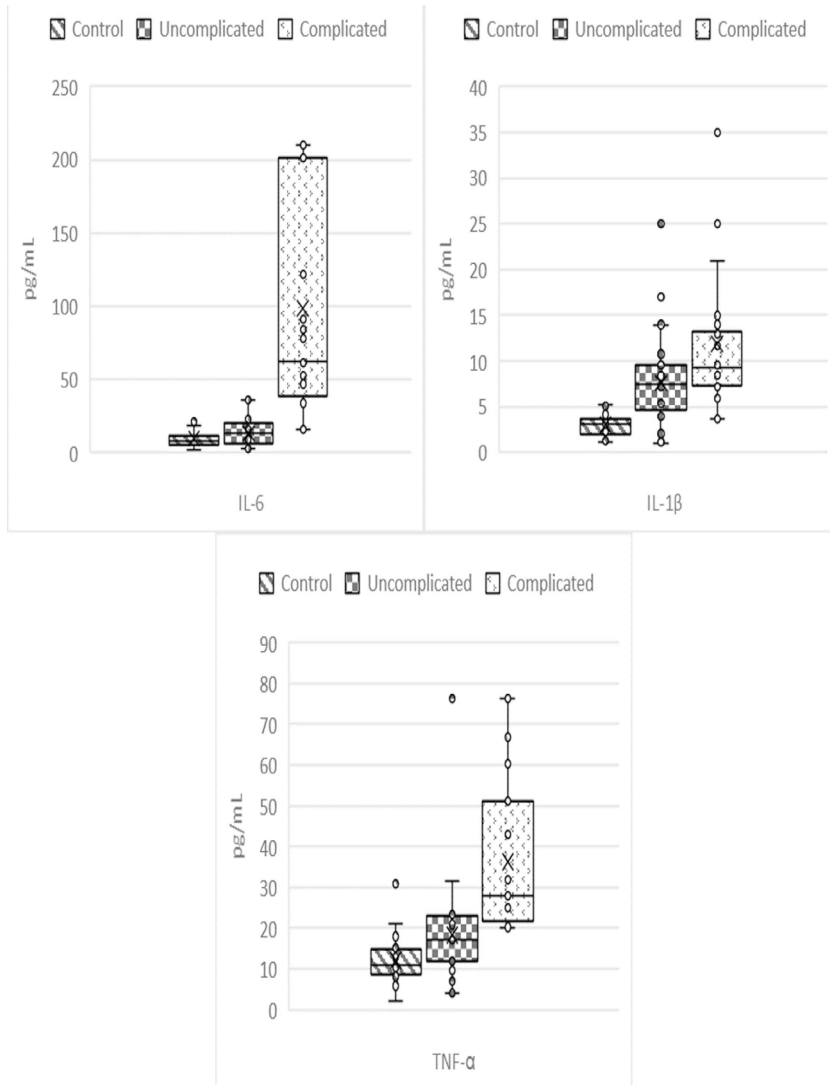

Figure 1. Comparisons of interleukin 6, interleukin 1 beta, and tumor necrosis factor-alpha levels between the control, uncomplicated, and complicated acute appendicitis groups.

acute appendicitis. Cut-off points of WBC, CRP, NLR, IL-6, IL-1β and TNF-α were calculated as 13.5 $\times 10^3/\mu L$, 1.92 mg/dL, 6.09, 23.4 pg/mL, 5.6 pg/mL and 24 pg/mL, and the values higher than these cut-off points were correlated significantly with the presence of complicated acute appendicitis (p=0.0014, p<0.001, p=0.009, respectively and p<0.001 for the rest). When AUCs of these biochemical parameters were compared (Figure 2), a significant difference was found in the predictive power of IL-6 compared with all other parameters (p<0.001 for IL-1β, WBC and NLR, p<0.01 for TNF-α and CRP). A significant difference was also found in the predictive power of IL-1β compared with IL-6 and TNF-α (p<0.001 and p<0.01, respectively). A significant difference was also found in the predictive power of TNF-α compared with IL-6, IL-1β, WBC (p<0.01 for all) and NLR (p<0.001). AUC of IL-6 was larger than AUCs of all other parameters, suggesting the highest predicting power of IL-6 among other parameters.

DISCUSSION

Appendicitis is the most common reason of emergency cases including the abdominal surgeries (Di Saverio et al. 2020). The diagnosis of the cases generally involves the patient history, clinical symptoms and the findings of physical examination and biochemical tests. As the atypical clinical symptoms are confused with other organ damages especially in women and children, the diagnostic accuracy becomes challenging and time-consuming. Although radiological analysis reduces the incidence of the negative appendectomy, it may also be time-consuming and over costing in emergency cases (Buyukbese Sarsu & Sarac 2016). In addition to the need of the appendectomy, AA may give rise to a complicated appendicitis if the diagnosis period prolongs, leading to long duration of hospitalization and financial burden on both patient and social security institutions. Although there are many biochemical and inflammatory parameters such as WBC, CRP and NLR to detect the acute appendicitis, still, the ratio of false positivity in diagnosis was reported as 15% (Chen et al. 2016, Guller et al. 2011, Ozer et al. 2018, Ibrahim et al. 2020). A recent review stated that

Table II. Comparison of the clinical parameters of the patients with uncomplicated and complicated acute appendicitis.

		Uncomplicated AA (n=66)	Complicated AA (n=34)	P value
USG Diameter (mm) Presence of PF	X ± SD Range N (%)	8.57 ± 1.69 [6.2-14.0] 6 (9.1)	9.83 ± 1.8* [7.0-12.0] 23 (67.6)	**0.011** **< 0.0001**
Hypertension	N (%)	4 (6.1)	6 (17.6)	0.140
Diabetes	N (%)	3 (4.5)	4 (11.8)	0.354
Respiratory Disorders	N (%)	6 (9.1)	0 (0)	0.171
ASA grade I II III	N (%)	52 (78.8) 14 (21.2) 0 (0)	26 (76.5) 7 (20.6) 1 (2.9)	0.992

*p<0.05, **p<0.01, ***p<0.001 vs uncomplicated AA group. X ± SD: Mean ± Standard deviation, USG: Ultrasonography, PF: Periappendiceal Fluid.

traditional biomarkers (such as WBC, CRP) had a moderate diagnostic accuracy (0.75) but lower costs in the diagnosis of acute appendicitis. Conversely, novel markers (pro-calcitonin, IL-6 and urinary 5-HIAA, amyloid, omentin) were found to have high process-related costs including analytical times, but improved diagnostic accuracy (Acharya et al. 2017, Sit et al. 2014). Therefore, new biomarkers have been investigated for an accurate and rapid diagnosis of the disease. That is to say that the aim of the current study was to reveal the predictive value of proinflammatory and inflammatory cytokines, which have been investigated in various inflammatory diseases, including the differential diagnosis of complicated AA.

The biochemical diagnostic criteria for AA are controversial, therefore, attempts to identify AA-specific biomarkers have significantly increased over the last decade. Several cytokines such as IL-6, IL-1β, and TNF-α have been the subject of multiple recent studies (Stankovic et al. 2019, Andersson et al. 2014) but, the overall accuracy of testing these cytokines remains to be determined. Thus, in the present study, the diagnostic value of serum IL-6, IL-1β, and TNF-α levels to predict both the presence of AA and the discrimination of uncomplicated and complicated AA was evaluated and higher levels of these cytokines were found in patients with the complicated AA compared to the control and uncomplicated patients.

Although many attempts to identify appendicitis-specific biomarkers have focused on individual proteins, it is not possible that a single cytokine will be a definitive diagnostic marker for AA since the etiological causes of the disease is diverse (Naqvi et al. 2019). In the present study, the diagnostic accuracy, sensitivity, and specificity of IL-6 were higher than of IL-1β, TNF-α, WBC, CRP, and NLR in predicting the complicated acute appendicitis. TNF-α also showed a higher accuracy and specificity than IL-1β, WBC, CRP, and NLR while IL-1β showed a lower diagnostic value than all other parameters, although its serum levels in the patients with complicated AA were significantly higher than the patients with uncomplicated AA.

A study was analyzed the diagnostic role of serum cytokines depends on the etiology and pathogenesis of AA and acute mesenteric lymphadenitis (AML) and revealed that AUC was 0.77 for IL-6 with the cut-off value of 4.3 pg/mL for AA with a sensitivity of 67.7% and a specificity of 76.9% (p=0.001) in discriminating between AA and AML. They also presented the WBC concentration in blood with cut-off value ≥10.7x103/µL for AA, reflecting the AUC value of 72% in ROC analysis, sensitivity of 74.2% and specificity of 53.8%. They concluded that IL-6 is more specific for the diagnostics of AA than

Table III. Comparison of the postoperative parameters of the patients with uncomplicated and complicated acute appendicitis.

		Uncomplicated AA (n=66)	Complicated AA (n=34)
Hospitalization (day)	X ± SD [Range]	1.39 [1-3]	3.56 [1-7]
Postop Infection	N (%)	2 (3.03)	10 (29.4)
Postop Intraabdominal Abscess	N (%)	1 (1.5)	6 (17.6)
Postop Fistula	N (%)	0 (0)	2 (5.9)
Re-hospitalization	N (%)	0 (0)	6 (17.6)

X ± SD: Mean ± Standard deviation.

WBC because it reduces the possibility of false-positive case detection, however, they do not support the use of IL-6 measurement alone as a substitute for the clinical, routine laboratory and radiological examinations in patients with suspicion for AA (Zviedre et al. 2016). In the present study, ROC analysis gave a highly significant diagnostic value of IL-6 in predicting the disease, with a cut-off value as 23.4 pg/mL. Contrary to descriptive and comparative statistical methods, ROC curve analysis allows evaluation of appropriateness of diagnostic parameters and diagnostic accuracy, allowing to evaluate the likelihood that a case with a given test result has that disorder (Buyukbese Sarsu & Sarac 2016). The large difference between cut-off value and other cut-off values in the literature for IL-6 is probably due to small size of the group, in addition to the absence of any power analysis to detect the effective sample size. On the other hand, some of the results of these studies were obtained by comparing AA patients with controls, and in the present study, patients with complicated and uncomplicated AA were compared. This may be one of the main reasons for different cut-off values and different results. In another study included pediatric patients revealed that comparing complicated AA and uncomplicated AA, IL-6 was the only biomarker of significance yielding 77.4% sensitivity and 58.1% specificity with a 26.43 pg/ml cutoff value. Serum IL-6 in complicated AA was five times higher than the control group and three times higher than uncomplicated AA. This difference in IL-6 levels between complicated AA and uncomplicated AA can determine the severity of appendicitis (Kakar et al. 2020). In our study, we gave the effect size for IL-6, IL-1β and TNF-α parameters as 0.78 with 0.05 Type I error rate, and 90% power. As the minimum

Table IV. Comparative diagnostic test results in predicting the complicated acute appendicitis.

	Diagnostic Scan					ROC Curve	
	Cut off	Sensitivity [95% CI]	Specificity [95% CI]	PPV [95% CI]	NPV [95% CI]	AUC [95% CI]	P*
WBC (x10^3/μL)	13.5	73.5 [55.6-87.1]	63.6 [50.9-75.1]	51.0 [36.3-65.6]	82.4 [69.1-91.6]	0.678 [0.57-0.79]	**0.0014**
CRP (mg/dL)	1.92	88.2 [72.5-96.7]	71.2 [58.7-81.7]	61.2 [46.2-74.8]	92.2 [81.1-97.8]	0.815 [0.72-0.91]	**<0.001**
NLR	6.09	67.65 [49.5-82.6]	63.64 [50.9-75.1]	48.9 [34.1-63.9]	79.2 [65.9-89.2]	0.652 [0.54-0.77]	**0.009**
IL-6 (pg/mL)	23.4	91.18 [76.3-98.1]	96.67 [89.5-99.6]	93.9 [79.8-99.3]	95.5 [87.5-99.1]	0.968 [0.93-1.00]	**<0.001**
IL-1β (pg/mL)	5.6	51.18 [46.3-68.1]	45.45 [33.1-58.2]	46.3 [34.0-58.9]	90.9 [75.7-98.1]	0.697 [0.59-0.80]	**<0.001**
TNF-α (pg/mL)	24	73.53 [55.6-87.1]	95.45 [87.3-99.1]	89.3 [71.8-97.7]	87.5 [74.6-93.6]	0.874 [0.80-0.95]	**<0.001**

*p<0.05 is the significance level. ROC: Receiver operating characteristic, PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: Area under ROC curve, CI: Confidence Interval, WBC: White blood cells, CRP: C-reactive protein, NLR: Neutrophil-Lymphocyte ratio, IL-6: Interleukin 6, IL-1β: Interleukin 1 beta, TNF-α: Tumor necrosis factor-alpha.

needed numbers of cases were determined, we collected the data of 20 control cases, 66 cases of uncomplicated and 34 cases of complicated AA. Interestingly, IL-6 showed the largest AUC and highest accuracy, sensitivity and specificity in predicting the complicated AA among all biochemical parameters investigated. Therefore, these results reveal the importance of studying serum inflammatory markers in AA that could be helpful to differentiate the type of AA because most patients are healthy before developing AA, and the type of symptoms before hospitalization is typically similar among uncomplicated and complicated AA.

The most used laboratory markers for reinforcing the diagnosis of AA are still WBC, CRP and NLR. High WBC, CRP and NLR levels have been reported to be associated with the other laboratory biomarkers and imaging modalities, hence, useful for the diagnosis of the complicated appendicitis (Yamashita et al. 2016, Huckins & Copeland 2020, Fatima et al. 2021). However, there are also controversy findings which failed to determine the difference between the uncomplicated and complicated appendicitis patients regarding WBC and CRP levels (Ozer et al. 2018). Some studies claimed that only CRP without combination the other
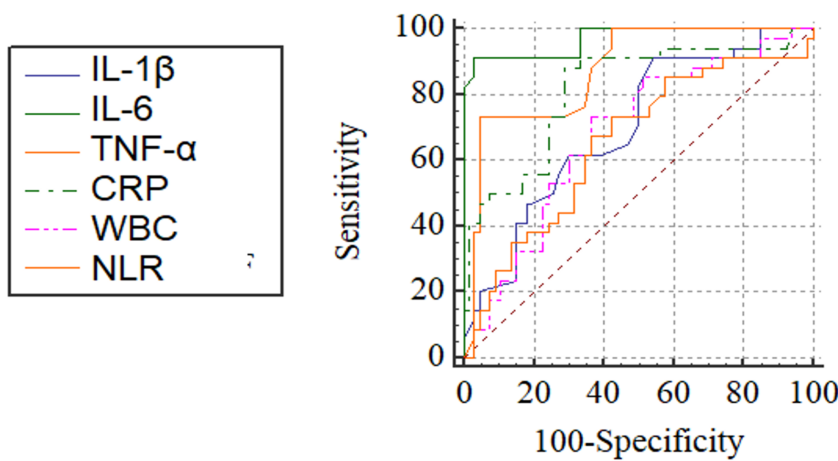

Figure 2. Receiver operating characteristic (ROC) curve analysis for the diagnosis of patients with complicated acute appendicitis using a WBC: White blood cells, CRP: C-reactive protein, NLR: Neutrophil-Lymphocyte ratio, IL-6: Interleukin 6, IL-1β: Interleukin 1 beta, TNF-α: Tumor necrosis factor-alpha.

	ΔAUC	95%CI	P value
IL-6 ~ IL-1β	0.271	0.169 - 0.373	**<0.0001**
IL-6 ~ TNF-α	0.094	0.025-0.164	**0.007**
IL-6 ~ CRP	0.153	0.061-0.245	**0.001**
IL-6 ~ WBC	0.219	0.178-0.403	**<0.0001**
IL-6 ~ NLR	0.316	0.202-0.430	**<0.0001**
IL-1β ~ TNF-α	0.177	0.060-0.293	**0.003**
IL-1β ~ CRP	0.118	-0.0003-0.236	0.051
IL-1β ~ WBC	0.019	-0.124-0.163	0.791
IL-1β ~ NLR	0.045	-0.101-0.191	0.548
TNF-α ~ CRP	0.059	-0.043-0.160	0.257
TNF-α ~ WBC	0.196	0.071-0.321	**0.002**
TNF-α ~ NLR	0.221	0.093-0.350	**<0.001**

markers estimation did not improve accuracy in the diagnosis of acute appendicitis (Du et al. 2020, Huckins & Copeland 2020). For a long time, blood WBC, CRP, and NLR tests have been used for the diagnosis of appendicitis, but their sensitivities and specificities are varied. In literature, the sensitivity of WBC has been reported to vary between 19% and 90%, and the specificity between 44% and 100% (Buyukbese Sarsu & Sarac 2016, Huckins & Copeland 2020, Yu et al. 2013). In the present study, we reported the accuracy, sensitivity, and specificity of WBC as 67.8%, 73.5%, and 63.6%, respectively, which are obviously in the range of literature. However, IL-6 and TNF-α showed higher accuracy, sensitivity, and specificity than WBC, suggesting a more valuable diagnostic tool in detecting complicated AA.

In AA, migration of leukocytes to target tissues results in release of cytokines like CRP. The synthesis of CRP increases within 4–6 hours after acute tissue injury or onset of the inflammation and doubles every 8 hours thereafter peaking at nearly 36–50 hours. Since its half-life is only 4–7 hours, its concentration rapidly drops. Therefore, in patients whose symptoms manifest within less than 12 hours, it has a relatively lower sensitivity. The sensitivity and specificity of CRP have been shown to vary between 48% and 98.7% and 57% and 82%, respectively (Buyukbese Sarsu & Sarac 2016). In the present study, we reported them as 88.2% and 71.2%, respectively, showing a consistency with the literature. Buyukbese Sarsu & Sarac, suggested that combined use of cut-off values of WBC (≥13.1x10^3/mL) and CRP (≥1170 µg/L) yields a higher sensitivity and NPV for the diagnosis of complicated appendicitis (Buyukbese Sarsu & Sarac 2016). Similarly, in the present study, cut-off points of WBC and CRP were calculated as 13.5 x10^3/µL and 1.92 mg/dL, respectively. These similarities with the literature offer that

WBC and CRP are indispensable markers of AA that should be examined in routine triage of the patients, however, given that IL-6 had higher diagnostic features than WBC and CRP, it would probably reasonable to refer to this cytokine for differential diagnosis of complicated AA patients.

A study showed that NLR of 5.74 was significantly associated with complicated AA (Kahramanca et al. 2014). The sensitivity and specificity of NLR were 70.8% and 48.5%, respectively. In a similar manner, our ROC analysis gave a cut-off value of 6.09 with a 67.65% sensitivity and 63.64% specificity in predicting the complicated AA. Although the size of population involved in the study was large, they did not gave the effect size of population and samples (Kahramanca et al. 2014), therefore the dissimilarities are expected for the cut-off values and other diagnostic features of NLR. Interestingly, in our study, NLR has the lowest accuracy, sensitivity and specificity in predicting the complicated AA although its serum level was significantly increased in these patients compared with the uncomplicated AA.

Complicated AA is related to a variety of potentially serious complications like infection, intraabdominal abscess or fistula formation, small bowel obstruction, leading to re-hospitalization of the patient. In the present study, the patients of complicated AA were hospitalized significantly longer than the patients with uncomplicated one. In addition, more incidence of postoperative infection, formation of the intraabdominal abscess and fistula and re-hospitalization were recorded in the complicated group. Thus, the early appendectomy remains the gold standard, and discrimination of complicated appendicitis from uncomplicated one is vital to avoid delays of essential operative procedures for these patients (Blakely et al 2011, Kaiser et al. 2018).

The results of the another trial identify 2 out of 20 tested serum cytokines including our tested cytokines, IL-10, MIP-1α, which showed statistically different concentrations between pathohistological groups (uncomplicated, complicated and normal appendix-early stage appendicitis). IL-10 was the only interleukin whose pre-operative serum level varied significantly between uncomplicated and complicated appendicitis. MIP-1α differentiated uncomplicated and complicated in a similar manner as IL-10 on the third postoperative day. Unlike to our results, there was no differences neither preoperative nor postoperative IL-1β, TNF-α and IL-6 levels between groups (Stankovic et al. 2019).

In this study, although IL-6, IL-1β, and TNF-α levels were not evaluated in the diagnosis of whether the patient had appendicitis, we thought that by adding serum IL-6, IL-1β, and TNF-α to the combination of WBC, CRP, and NLR, the diagnostic accuracy in terms of the severity of acute appendicitis could be increased. We interpret from these results that if the diagnostic roles of these cytokines are understood well and used to differentiate the complicated acute appendicitis, they will be useful in predicting the future complications that the patient may experience prognostically and that antibiotic therapy or interventional procedures to be applied to the patient can be planned and performed in this context. Such as, the surgical team could be organized and with the adequate equipment to perform all the procedure laparoscopically diminishing the risk of conversion or other surgical problems that may arise such as not enough water to wash-out the abdomen and diminish the risk of an intraabdominal abscess. Surgical preparation can be done more carefully in the cases suspected to be complicated due to cytokine levels. For example, to prevent intraabdominal abscess formation, we can keep more liquid for use in washing. By washing with more fluid, we can reduce the complication rate in the treatment of perforated appendicitis by reducing the bacterial organisms and other contaminants that cause peritonitis in the infected peritoneal cavity (Ohno et al. 2004). In cases with uncomplicated appendicitis detected in the early period, a gram (+) based antibiotherapy is primarily applied as prophylactic while in cases with complicated acute appendicitis, the broad-spectrum antibiotics can be given due to the increases in biomarker levels mentioned in our study, therefore, a possible intra-abdominal septic entity can be prevented beforehand. Further studies are needed to investigate new biomarkers and address concerns over bias, in order to improve the diagnosis of complicated acute appendicitis.

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