A combination of preoperative serum direct bilirubin and neutrophil–lymphocyte ratio can discriminate cases of complicated appendicitis

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Objectives
Evaluation of the ability of routine investigations for patients presenting with acute right lower abdominal pain for preoperative prediction of complicated appendicitis (CA).

Patients and methods
In all, 311 patients who had acute appendicitis were evaluated clinically and by abdominal ultrasonography and obtained blood samples for the estimation of serum C-reactive protein (CRP), direct bilirubin (DB), determination of total leukocyte count, and for the calculation of neutrophil/lymphocyte ratio (NLR). Patients were categorized according to operative findings as CA or uncomplicated appendicitis (UCA). The diagnostic performance of the estimated variables was statistically evaluated as predictors for CA.

Results
Patients who had CA were mostly males and older than those who had UCA. Preoperative abdominal ultrasonography diagnosed mild, moderate, and severe acute appendicitis in 138, 136, and 37 cases, respectively. Operative findings defined 48 patients had CA and 263 as having UCA. Preoperative total leukocyte count and NLR, and serum CRP and DB were significantly ($P<0.001$) higher in CA than in UCA patients. Statistical analyses defined high serum DB, high NLR, and high serum CRP as positive preoperative predictors for CA in the decreasing order of significance. Kaplan–Meier analysis defined serum DB level at 0.51±0.03 mg/dl [95% confidence interval (CI): 0.471–0.589], NLR at a level of 4.7±0.04 (95% CI: 4.62–4.84), serum CRP at 13±1.6 (95% CI: 12.3–13.5) as predictors for CA in decreasing positive predictive and specificity values. The percentage of true positive cases of CA in comparison to operative findings was 79.2% on using DB and NLR, 72.9% on using a combination of CRP and NLR, 68.8% with DB and CRP combination, and 87.5% with a combination of DB, CRP, and NLR.

Conclusion
Preoperative routine laboratory investigations can discriminate against CA cases. Combined estimation of DB and determination of NLR with or without estimation of serum CRP provided a high discriminative ability for CA cases.

Introduction

Acute appendicitis (AA) is one of the most common causes of acute abdomen and represents the most common surgical emergency that affects all age groups and no sex is exempted [1]. The diagnosis of AA mostly depends on clinical judgment by assessment of the clinical presentation and physical findings and may be supplemented by laboratory tests and ultrasonography. However, confirmation of the diagnosis depends solely on the pathological diagnosis of the resected appendicular specimen [2].

The recognition and management of patients presented with acute right lower abdominal pain at extremes of age are more complicated than in adult

patients [3]. Moreover, negative appendectomy, which is still a challenge, accounts for an incidence of 15–39% of cases presenting by acute right lower abdominal pain [4]. This high incidence rate of negative appendectomies could be attributed to being misdiagnosed with other conditions such as lymphoid hyperplasia [5] or Henoch–Schonlein purpura [6].

C-reactive protein (CRP) is an evolutionary highly conserved member of the pentraxin superfamily of

proteins. It is synthesized by the liver with a rapid increase in its synthesis rate within hours after tissue injury or infection [7]. CRP contributes to host defense and is a part of the innate immune response [8].

Objectives
This work aimed to evaluate the discriminative ability of an array of routine preoperative investigations for patients presenting clinically with acute right lower abdominal pain.

Patients and methods

Design
Prospective comparative study.

Setting
Department of General Surgery, Benha Teaching and University Hospitals in conjunction with multiple private surgical centers.

Ethical considerations
The preliminary approval of the study protocol was obtained before the start of the study in June 2021 and the final approval was obtained after completion of case collection in October 2022.

Participants
All patients presenting to the emergency department at the participating hospitals with acute right lower abdominal pain were clinically evaluated for enrolment requirements. Patients will be evaluated clinically, by abdominal ultrasonography imaging and given blood samples for estimation of the assigned investigations.

Exclusion criteria
Exclusion criteria were pregnancy, presence of liver, renal or cardiac diseases, autoimmune diseases, maintenance on treatment for peptic ulcer, cholecystitis or inflammatory bowel diseases, immunosuppressive drugs, definite gynecological problems especially ectopic pregnancy, and tubo-ovarian diseases.

Inclusion criteria
Acute right lower abdominal pain and absence of exclusion criteria are the inclusion criteria. No age or sex was excluded.

Laboratory investigations
Venous blood samples were collected aseptically for the estimation of hemoglobin concentration, total leukocyte count (TLC), and differential count of white blood cells for the calculation of neutrophil/lymphocyte ratio (NLR) and estimation of serum CRP and direct bilirubin (DB). All excised specimens were sent for pathological examination.

Grouping
Patients were categorized as complicated appendicitis (CA) or uncomplicated appendicitis (UCA) according to operative findings.

Study outcomes
The primary outcome is the ability of the estimated preoperative laboratory variate as a predictor for the presence of CA.

The secondary outcome is the diagnostic performance of the estimated laboratory variate in various combinations for discrimination between CA and UCA cases.

Statistical analysis
Demographic and laboratory data of the enrolled patients were compared between both groups using the one-way analysis of variance and χ^2 tests. The predictability of the obtained data for CA cases was evaluated using the receiver-operating characteristic curve and regression analysis. Kaplan–Meier regression analysis was used to identify the cutoff point for each variate at which the hazard risk for having CA was increased by 50%, and these cutoff points were used to determine the test performance characters. Statistical analyses were conducted using the SPSS (Version 22, 2015; Armonk, USA) Software program (IBM, USA). The P value at a cutoff point of less than 0.05 indicates the significance of the results.

Results
During the study duration, 348 patients were evaluated; 37 patients were excluded for the detection of gynecological problems (n=11), chronic calcular cholecystitis (n=10), maintenance on the treatment of peptic ulcer disease (n=7), hepatitis (n=5), ulcerative colitis (n=2), and rheumatic diseases (n=2). In all, 311 patients were enrolled in the study (Fig. 1).
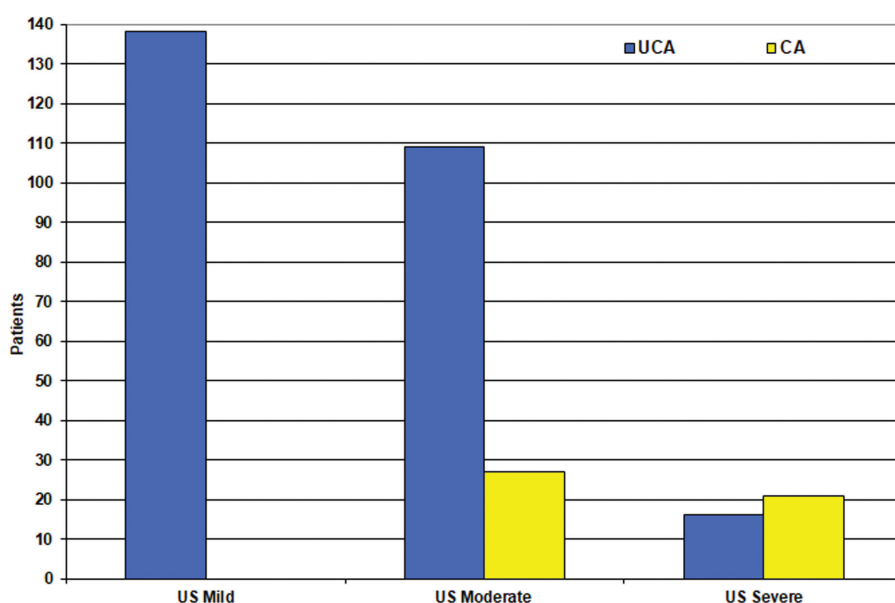
Preoperative abdominal ultrasonography defined 138 cases had mild AA (44.4%), 136 cases had moderate AA (43.7%), and 37 (11.9%) cases had severe AA. Intraoperative diagnosis defined 48 (15.4%) cases had CA and 263 (84.6%) cases as UCA (Figs 1 and 2). The frequency of the male sex was significantly higher among patients who had CA than among patients

Figure 1
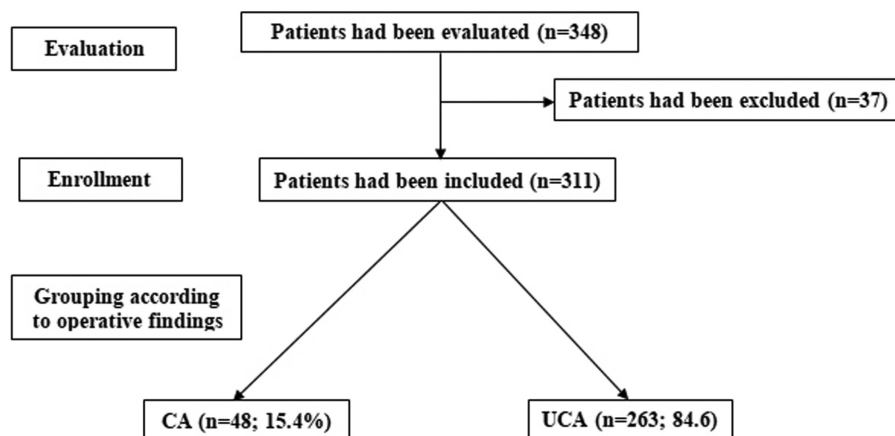
Patients' flowchart.

Figure 2

Patients' distribution according to preoperative US versus intraoperative diagnoses. US, ultrasonography.

who had UCA (58.3 vs. 40.1%; *P*=0.026). The mean age of patients who had CA (40.5±13.7 years) was significantly (*P*=0.004) higher than the mean age of patients who had UCA (35.4±10.6 years).

Patients who were found to have CA had nonsignificantly lower hemoglobin concentration than patients who were found to have UCA. On the contrary, patients of the CA group had significantly (*P*<0.001) higher TLC and NLR, and higher serum CRP and DB compared with patients of the UCA group (Table 1).

The receiver-operating characteristic curve analysis stratified the at-admission data of enrolled patients as positive predictors for CA according to the

Table 1 At-admission laboratory investigations of patients of the studied groups

| Laboratory variables	UCA group (N=263)	CA group (N=48)	P value
Hemoglobin concentration (g/dl)	13.3±1.5	12.9±1.2	0.06
TLC (10^3 cell/ml)	11.2±1.36	13.06±1.72	<0.001
NLR	3.83±0.47	4.34±0.5	<0.001
CRP (mg/l)	9.44±2.2	11±2.6	<0.001
Direct bilirubin (mg/dl)	0.31±0.09	0.45±0.13	<0.001

CA, complicated appendicitis; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio; TLC, total leukocyte count; UCA, uncomplicated appendicitis. *P* value less than 0.05 indicates a significant value.

significance of the AUC as follows: high DB, high NLR, TLC, serum CRP, old age, and male sex

(Fig. 3). Regression analysis for the at-admission data defined high serum DB, high NLR, old age, and high serum CRP as positive predictors for CA in the decreasing order of significance (Table 2).

Kaplan–Meier analysis to define the cutoff points of the predictors for the presence of CA defined serum

Figure 3
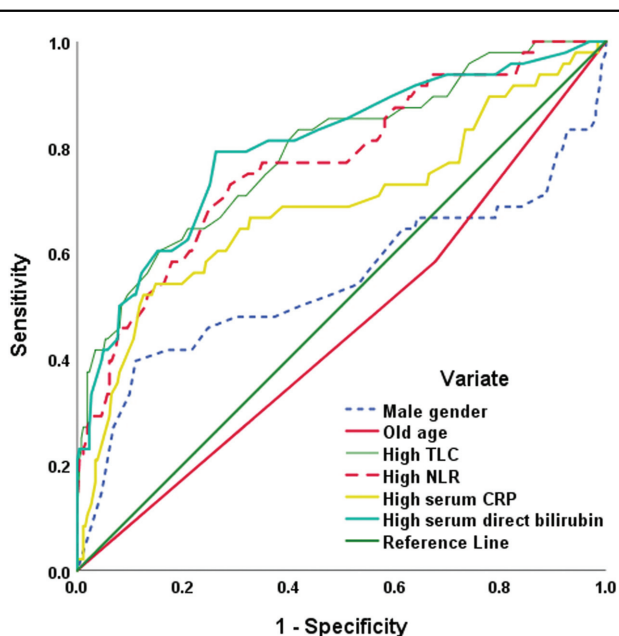
ROC curve analysis of at-admission variate as predictors for the presence of CA. CA, complicated appendicitis; ROC, receiver-operating characteristic.

level of DB at 0.51±0.03 mg/dl (95% CI: 0.471–0.589), NLR at a level of 4.7±0.04 (95% CI: 4.62–4.84), serum CRP at 13±1.6 (95% CI: 12.3–13.5), and mean age of 51±0.5 years (95% CI: 49.3–51.4%) in decreasing positive predictive and specificity values (Table 3, Fig. 4a–d).

The percentage of true positive cases of CA in comparison to operative findings was 79.2% on the application of preoperative combined levels of DB and NLR, 72.9% using the preoperative combination of CRP and NLR, 68.8% with the use of a preoperative combination of DB and CRP, and was 87.5% with a combination of preoperative levels of serum DB and CRP and NLR (Fig. 5).

Discussion

Estimation of serum bilirubin is not one of the routine investigations for cases suspicious to have acute appendicitis (AA), especially DB. However, the results of this study detected high serum levels of DB in cases of CA than in UCA. Statistical analyses defined the high discriminative ability of serum DB over other clinical or laboratory investigations for cases of CA. These findings go hand in hand with Shuaib et al. [9], who found that serum total bilirubin (TB) levels were significantly higher in CA than in UCA cases. Also, Feng et al. [10] found that the diagnostic performance of a combination of the duration of abdominal pain, clinical suspicion of the presence of

Table 2 Statistical analyses of at-admission data of enrolled patients as predictors for complicated appendicitis

| Analyses variate	ROC curve analysis			Regression analysis	
	AUC±SE	P value	95% CI	β	P value
Age	0.546±0.057	0.312	0.434–0.657	0.163	0.001
Male sex	0.453±0.046	0.303	0.363–0.543	0.046	0.969
TLC	0.770±0.039	<0.001	0.716–0.866	0.091	0.368
NLR	0.791±0.038	<0.001	0.693–0.847	0.319	<0.001
CRP	0.687±0.048	<0.001	0.592–0.782	0.178	0.013
Direct bilirubin	0.801±0.038	<0.001	0.726–0.875	0.337	<0.001

AUC, area under the curve; CI, confidence interval; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio; ROC, receiver operating characteristic; TLC, total leukocyte count; β, regression coefficient. P value less than 0.05 indicates a significant value.

Table 3 Test validity characters of the studied variate as predictors for the presence of complicated appendicitis

| Variate (cutoff points)	Sensitivity rate [n (%)]	Specificity rate [n (%)]	Positive predictive value [n (%)]	Negative predictive value [n (%)]	Accuracy rate [n (%)]
Age (51±0.5) (years)	34.2 (95% CI: 19.6–51.4%)	93.4 (95% CI: 89.8–96%)	41.9 (95% CI: 27.8–57.5%)	91.1 (95% CI: 89–92.8%)	86.2 (95% CI: 81.8–89.8%)
NLR (4.7±0.04)	29.2 (95% CI: 17–44%)	97.3 (95% CI: 94.6–98.9%)	66.7 (95% CI: 46–82.4%)	88.2 (95% CI: 86.3–90%)	86.8 (95% CI: 82.5–90.4%)
CRP (13±1.6 mg/l)	20.8 (95% CI: 10.5–35%)	96.2 (95% CI: 93.1–98.2%)	50 (95% CI: 30.6–69.4%)	86.9 (95% CI: 85.2–88.5%)	84.6 (95% CI: 80–88.4%)
Direct bilirubin (0.51 mg/dl)	39.6 (95% CI: 25.8–54.7%)	100 (95% CI: 98.6–100%)	100	90 (95% CI: 87.8–91.9%)	90.7 (95% CI: 86.9–93.7%)

CI, confidence interval; CRP, C-reactive protein; NLR, neutrophil/lymphocyte ratio.

Figure 4
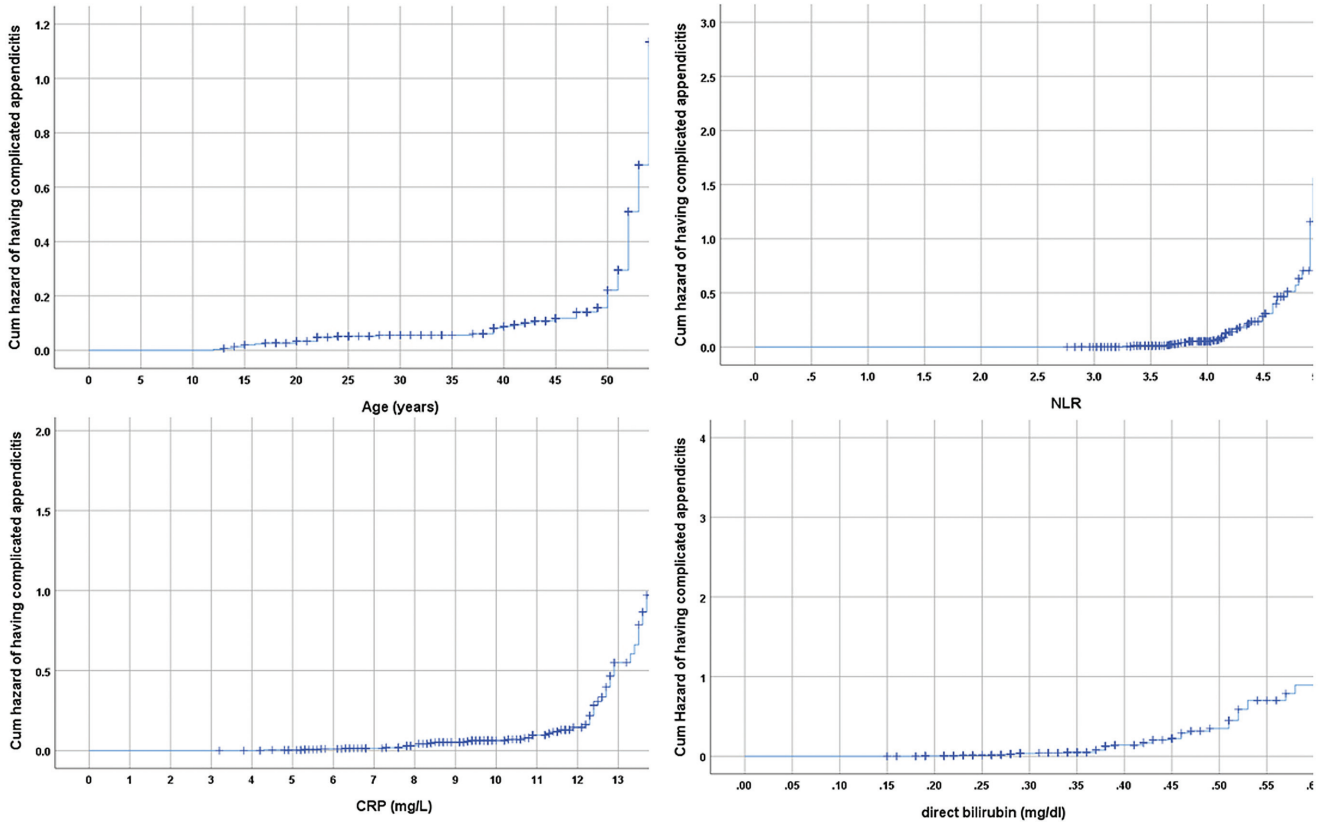
Cumulative hazard of having complicated appendicitis of the studied variate at their probable cutoff points as defined by Kaplan–Meier analysis.

Figure 5
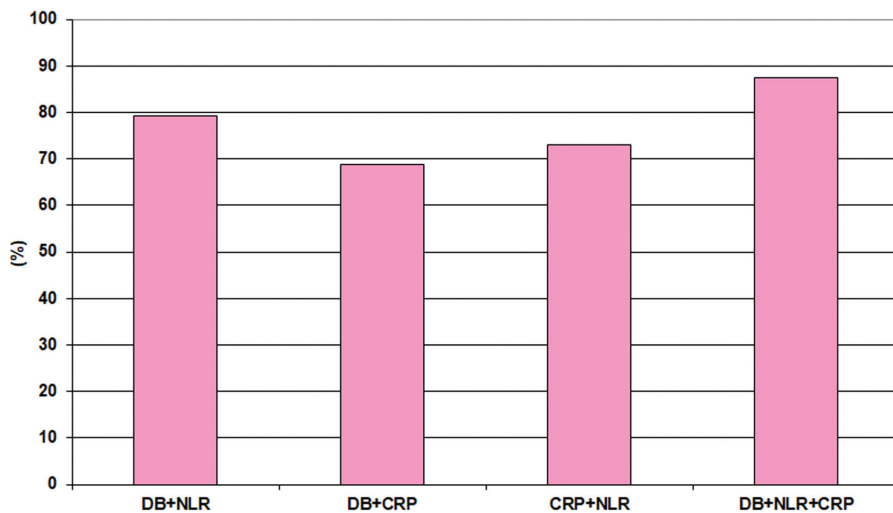
Percentage of true positive cases of CA according to various combinations of the estimated laboratory variate in comparison to operative findings. CA, complicated appendicitis.

peritonitis, and high serum bilirubin in elderly patients was high even after excluding another variate. In pediatric patients, Zhan *et al.* [11] provided a diagnostic model for differentiation between AA and Henoch–Schonlein purpura depending on the estimation of DB, neutrophil percentage, serum albumin and CRP, and platelet count, and the model showed high discriminative value, irrespective of the presence of fever and Koirala *et al.* [12] detected significantly (*P*<0.001) higher serum total and DB in

children with CA than UCA, and statistical analyses defined high sensitivity and specificity rates for high serum bilirubin for the diagnosis of CA.

The detected high NLR in CA than in UCA cases assured its diagnostic value for CA and NLR at a cutoff point of less than 4.7. NLR showed a high negative predictive value, but values greater than 4.7 had medium positive predictive value for CA. Similarly, Gurger and Telo [13] and Delgado-Miguel et al. [14] found that NLR was significantly higher in AA patients than in healthy volunteers. Moreover, Kubat and Şengül [15] documented the ability of leucocyte parameters to differentiate between patients who had CA and those who had UCA. Further, Delgado-Miguel et al. [14] found that NLR at a cutoff point of 2.65 showed high sensitivity and specificity for discrimination between the presence and absence of appendicitis. In line with the suggested cutoff point (NLR=4.7), Esquivel-Esquivel and Horta-Baas [16] found that NLR at a cutoff point of 5 had sensitivity, specificity, positive predictive value, and negative predictive values of 85.1, 78.9, 79.5, and 84.6%, respectively.

However, NLR as the sole preoperative parameter could not be a reliable marker, but its combination with estimated serum DB improved the diagnostic yield of NLR. This assumption goes hand in hand with Esquivel-Esquivel and Horta-Baas [16], who documented that NLR alone is insufficiently accurate to confirm or rule out the presence of AA, but its use together with other tests improved the diagnostic yield of NLR. Also, Ayeni et al. [17] documented that both neutrophil and platelet/lymphocyte ratios are reliable, synergistic markers for the prediction of CA and can guide nonoperative management of patients presenting by pain simulating that of AA.

The estimated serum CRP levels were significantly higher in samples of patients who had CA than in samples of patients who had UCA and showed a medium diagnostic value for CA at a cutoff point of 13 mg/l. In line with these data, Blok et al. [18] reported that the estimation of serum CRP improves the diagnosis of AA in children depending on history and physical examination, and serum CRP at a cutoff point of 10 mg/l could diagnose AA with sensitivity and specificity rates of 87 and 77%, respectively. Also, Steiner et al. [19] documented that high serum CRP, TLC, large outer appendicular diameter, and old age are risk factors for recurrent AA after conservative treatment for children. Moreover, Kubota et al. [20]

concluded that an appendicolith larger than 10-mm in diameter or CRP level higher than 10 mg/l is a surgical indication.

Evaluation of the discriminatory value of the laboratory variate in reciprocal combinations detected the highest value for DB with NLR (79.2%), followed by NLR with CRP (72.9%) and DB with CRP (68.8%), and the combined three variates had a discriminative value of 87.5%. These findings spotlight the need for more variates for preoperative prediction of CA. Similarly, Patmano et al. [21] detected significantly high levels of TLC, NLR, serum CRP, and TB in cases that had perforated appendices. Also, Dinç et al. [22] found a combination of the direct neutrophil index and serum bilirubin can be used as laboratory parameters to distinguish between CA and UCA cases. Further, Shuaib et al. [9] combined high serum TB and low serum sodium levels and showed significant discriminatory value for the diagnosis of perforated/necrotizing appendicitis.

Conclusion
The utilization of routine laboratory investigations for preoperative diagnosis of AA and differentiation between CA and UCA is feasible, easy, and cheap, especially wherever resources are limited. Combined estimation of direct or even TB in combination with the determination of NLR and estimation of serum CRP provided a high discriminative ability for CA cases.

Conflicts of interest
There are no conflicts of interest.

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