Diagnostic Value of Serum Bilirubin and Liver Enzyme Levels in Acute Appendicitis

Avinoam Nevler MD[1,2,3,4]*, Yaniv Berger MD[2,4]*, Avital Rabinovitz MD[2,4#], Oded Zmora MD[2,4], Moshe Shabtai MD[2,4], Danny Rosin MD[2,4] and Mordechai Gutman MD FACS[2,4]

[1]Department of Surgery, Thomas Jefferson University, Philadelphia, PA, USA
[2]Department of Surgery and Transplantation and [3]Borenstein Talpiot Medical Leadership Program, 2012, Sheba Medical Center, Tel Hashomer, Israel
[4]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ABSTRACT: **Background:** Acute appendicitis (AA) is one of the most common indications for emergency abdominal surgery.

Objective: To assess the diagnostic and prognostic value of serum bilirubin and liver enzyme levels in the management of acute appendicitis.

Methods: Consecutive emergency department patients referred for a surgical consult for suspected AA were prospectively enrolled in the study. Data regarding demographic, clinical and laboratory results were recorded. Receiver operating characteristic (ROC) curve was performed for all evaluated parameters. Clinical and laboratory markers were evaluated for diagnostic accuracy and correlation to the clinical severity, histology reports, and length of hospital stay.

Results: The study was comprised of 100 consecutive patients. ROC curve analysis revealed white blood cell count, absolute neutrophil count (ANC), C-reactive protein, total-bilirubin and direct-bilirubin levels as significant factors for diagnosis of AA. The combination of serum bilirubin levels, alanine transaminase levels, and ANC yielded the highest area under the curve (0.898, 95% confidence interval 0.835–0.962, $P < 0.001$) with a diagnostic accuracy of 86%. In addition, total and direct bilirubin levels significantly correlated with the severity of appendicitis as described in the operative and pathology reports ($P < 0.01$). Total and direct bilirubin also significantly correlated with the length of hospital stay ($P < 0.01$).

Conclusions: Serum bilirubin levels, alone or combined with other markers, may be considered as a clinical marker for AA correlating with disease existence, severity, and length of hospital stay. These findings support the routine use of serum bilirubin levels in the workup of patients with suspected AA.

IMAJ 2018; 20: 176–181

KEY WORDS: appendectomy, appendicectomy, appendicitis, bilirubin, liver enzymes

The diagnosis of acute appendicitis can be challenging, and delayed diagnosis may lead to severe complications such as perforation and peritonitis, which are associated with high morbidity. Serum markers such as C-reactive protein (CRP), white blood cell count (WBC), serum bilirubin, and liver transaminase levels have been suggested as individual markers for appendicitis and appendiceal perforation [1,2].

In current practice, the diagnosis of acute appendicitis is mainly clinical, supported by laboratory and imaging studies. Ultrasonography and computed tomography (CT) may raise the diagnostic sensitivity to 66–100% and 90–100%, respectively, but these imaging practices entail several drawbacks such as cost, radiation exposure, and operator dependency [3]. Currently, no single clinical or laboratory test can determine if a patient has acute appendicitis. Non-inflamed appendix is still commonly found in the operating room.

The aims of this study were to assess the value of serum bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and WBC in diagnosing acute appendicitis and predicting its severity.

PATIENTS AND METHODS:

STUDY DESIGN

Patients who were referred for surgical evaluation for suspected or acute appendicitis in the emergency department were enrolled in this prospective cohort study.

SETTING AND PARTICIPANTS

The study was conducted in the emergency and surgical departments of a large tertiary medical center in Israel. Patient enrollment was between February 2012 and December 2012 and subsequent data collection was performed in 2013–2014. During the study period, patients presenting with right abdominal and/or right iliac fossa pain were routinely referred for surgical

*The first and second authors contributed equally to this study

#In partial fulfillment for the MD degree requirements at Sackler Faculty of Medicine, Tel Aviv University

Preliminary findings of this study were presented as an abstract at the Israeli Emergency Medicine Association (IEMA) Congress in Israel (March 2015)

Financial support
Dr. Nevler was supported by a research scholarship from the American Physician Fellowship

consult after initial assessment by a triage nurse or an emergency department (ER) physician. Routine laboratory studies were obtained in all patients, together with complete blood count and serum chemistry analysis, including bilirubin and transaminase levels. The exclusion criteria included age < 18 years and medical conditions that may affect liver enzyme levels such as cirrhosis, inflammatory bowel disease, any malignancy, pregnancy, and recent abdominal surgery.

DATA COLLECTION

Data regarding demographic, clinical, radiological, operative, and pathological features were analyzed. The Alvarado score [4] of each patient was calculated. Patients who were assessed as having acute appendicitis routinely underwent surgery, and acute appendicitis was defined as histological findings characteristic for acute appendicitis. Cases were defined as not having acute appendicitis (NAA) based on clinical, laboratory, and imaging findings, as well as a surgical consult that ruled out the diagnosis of acute appendicitis. In addition, cases initially suspected of acute appendicitis with histological findings of normal appendix were defined as NAA. All cases discharged from the ER, which were diagnosed as NAA, were reviewed for ER readmissions in the following week to rule out a possible false or missed diagnosis.

The severity of appendicitis was classified as normal (in cases of negative, normal appearing appendix), simple appendicitis, severe (phlegmonous/gangrenous appendix), and perforated appendicitis based on the operative report, which followed the classification systems described by Gomes and colleagues [5] and by Guzmán-Valdivia Gómez [6]. In a similar manner, the severity of appendicitis, as described in histological examination, was classified as normal, simple, severe (in cases of acute appendicitis with periappendicitis or phlegmonous/gangrenous), or perforated appendix similar to the pathologic classification described by Fallon and colleagues [7].

Length of hospital stay was recorded for all cases of acute appendicitis. Patients who were discharged from the ER were defined as length of hospital stay (LOS) = 0 days.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM Statistical Package for the Social Sciences statistics software, version 20 (SPSS, IBM Corp, Armonk, NY, USA). Categorical data are expressed as percentages and continuous data are expressed as mean ± standard deviation. Continuous data were compared by independent *t*-test and categorical data were compared by chi-square test or Fisher's exact test. A *P* value < 0.05 was defined as significant.

RECEIVER OPERATING CHARACTERISTIC ANALYSIS

In assessing the efficacy of a continuous parameter to be used as a discriminatory factor, the full spectrum of possible threshold values needs to be appreciated with regard to the resulting specificity and sensitivity. The receiver operating characteristic (ROC) analysis allows graphical plotting of the sensitivity vs. specify curve to assess the overall performance of the parameter as a diagnostic factor (area under the curve [AUC]) and discern the optimal threshold value. ROC curve analysis was performed for all evaluated parameters and corresponding AUCs with 95% confidence (95%CI) intervals were calculated. The statistically significant variables were inspected for a cutoff value for optimal diagnostic accuracy (calculated as [true positive + true negative]/N). Negative and positive predictive values (NPV, PPV) were calculated and recorded.

A multi-variable parameter combining the top significant markers from each marker group (inflammatory [WBC, ANC, CRP], jaundice [total bilirubin, direct bilirubin], and liver enzymes [AST, ALT, ALP]) was calculated and assessed using ROC analysis. Study population was grouped using the selected variable cutoffs and diagnostic accuracy of overall appendicitis, and severe appendicitis was assessed using Fisher's exact test. Correlation of the variables to the severity of appendicitis, according to operative and pathological reports, was assessed using Spearman's correlation test.

The study was approved by the institutional review board and all participants gave written informed consent. The study was registered at ResearchRegistry.com (UIN: researchregistry758). Reporting of the study findings was performed in accordance to the STROBE guidelines (http://strobe-statement.org/).

RESULTS

PARTICIPANTS AND DESCRIPTIVE DATA

During the study period, 100 consecutive patients were enrolled in the study (48 males, 52 females). Laparoscopic appendectomy was performed in 57 patients (4/57 cases had negative appendectomies, 1/57 appendectomy was converted to open). None of the 43 cases belonging to the NAA group had an ER readmission in the following 7 days resulting in diagnosis of acute appendicitis or periappendicular abscess. A summary of clinical presentation and laboratory tests is presented in Table 1. Mean total and direct bilirubin levels were 0.77 ± 0.58 mg/dl and 0.13 ± 0.11 mg/dl, respectively.

MAIN RESULTS

Absolute neutrophil count (ANC) > 8.23 was found to be the single most accurate diagnostic parameter (accuracy = 80%, $P < 0.01$). Direct and total serum bilirubin levels were also significantly diagnostic (accuracy of 74.6% and 73.7%, respectively). Although not statistically significant, ALT levels showed a statistical trend (accuracy = 59.3%, $P = 0.063$) in diagnosis of acute appendicitis. AST and ALP were both non-significant. ROC analysis is presented in Figure 1.

A combined multi-variable parameter was calculated as: MultiVar = ANC × total bilirubin × ALT.

Table 1. Comparison of clinical presentation and laboratory findings

N=100	AA (N=53)	NAA (N=47)	*P*
Age, years	29.3 ± 9.6	29.3 ± 9.6	NS
Gender: male, n	31 (58.5%)	17 (36.1%)	
Duration of symptoms, hours	27.6 ± 30.7	37.1 ± 40.1	NS
Maximal fever, °C	37.0 ± 0.69	36.9 ± 0.7	NS
Heart rate, beats/min	82 ± 16	83 ± 12	NS
Location of abdominal pain, n			
Right lower quadrant	40 (75.5%)	35 (74.5%)	
Periumbilical	5 (9.4%)	2 (4.3%)	
Suprapubic	4 (7.5%)	8 (17.0%)	
Diffuse	2 (3.8%)	0	
Other	2 (3.8%)	2 (4.2%)	NS
Laboratory tests (normal laboratory values)			
White blood cell count (4–10.8 k/ml)	14.61 ± 4.71	9.77 ± 3.64	***
Absolute neutrophil count (1.8–7.7 k/ml)	12.04 ± 0.47	6.71 ± 3.56	***
CRP (< 0.08–5 mg/l)#	37.40 ± 58.48	14.25 ± 23.54	NS
T.Bil (0.1–1.1 mg/dl)	0.97 ± 0.68	0.53 ± 0.30	***
D.Bil (0.0–0.3 mg/dl)#	0.18 ± 0.13	0.09 ± 0.04	***
AST (7–40 IU/L)	21.25 ± 7.79	20.83 ± 6.25	NS
ALT (7–45 IU/L)	23.02 ± 15.67	16.60 ± 6.06	*
ALP (45–115 IU/L)	75.67 ± 23.39	74.85 ± 22.38	NS
Alvarado Score	6.0 ± 2.2	3.6 ± 1.9	***

Overall imaging studies (%performed, %positive) N=100	
Abdominal sonography	53.0%, 43.4%
Computed tomography scan	34.0%, 67.6%
Either	75.0%, 56.0%

#>15% missing values, *Significant at the 0.05 level (2-tailed), **Significant at the 0.01 level (2-tailed), ***Significant at the 0.001 level (2-tailed)

AA = acute appendicitis, NAA = not acute appendicitis, CRP = C-reactive protein, T.Bil = total bilirubin, D.Bil = direct bilirubin, AST = aspartate transaminase, ALT = alanine transaminase, ALP = alkaline phospatase, NS = non significant

Table 2. Receiver operating characteristic analysis of clinical and laboratory markers

	AUC (95% confidence interval)	ROC *P* value	Cutoff value	Acc (%)	PPV (%)	NPV (%)	LR+
Alvarado Score	0.788 (0.696–0.880)	‹ 0.001	6.5	70.7%	92.0%	62.7%	10.5
Inflammatory							
White Blood Cell Count (k/ml^3)	0.805 (0.720–0.891)	‹ 0.001	14.6	69.0%	92.3%	60.8%	10.6
Abs. Neutrophil Count (k/ml^3)	0.837 (0.757–0.917)	‹ 0.001	12.7	69.0%	92.3%	60.8%	10.6
CRP (mg/L)	0.709 (0.587–0.831)	‹ 0.001	19.5	67.2%	66.7%	67.4%	2.6
Jaundice							
Total Bilirubin	0.791 (0.702–0.879)	‹ 0.001	0.73	69.7%	81.8%	62.9%	3.7
Direct Bilirubin	0.829 (0.731–0.927)	‹ 0.001	0.13	71.4%	78.3%	67.5%	3.7
Liver Enzymes							
Aspartate transaminase	0.536 (0.415–0.657)	0.56	17.0	58.4%	58.3%	59.1%	1.2
Alanine transaminase	0.617 (0.498–0.735)	0.063	27.0	57.0%	84.6%	52.1%	4.8
Alkaline phosphatase	0.486 (0.360–0.611)	0.819	103.0	50.1%	66.7%	48.7%	1.8
MultiVar	0.898 (0.835–0.962)	‹ 0.001	154.2	74.4%	96.2%	65.0%	21.7
Positive Abdominal Sonography		‹ 0.001		86.8%	87.0%	86.7%	7.8
Positive CT		‹ 0.001		94.1%	90.1%	95.6%	10.5

AUC = area under curve, ROC = receiver operator characteristics, Acc = accuracy, LR+ = positive likelihood ratio, CRP = C-reactive protein, MultiVar = absolute neutrofil count × total bilirubin × ALT, NPV = negative predictive value, PPV = positive predictive value

Italics = accuracy of imaging tests (current study) for comparison

An alternate MultiVar calculation based on direct bilirubin was also analyzed and found significant as having an AUC of 0.92; however, it was not further assessed due to a large number of missing values compared to total bilirubin values (37% vs. 1%). The combined parameter yielded an accuracy of 86.0% (PPV 85.4%, NPV 86.8%), similar to the efficacy of abdominal sonography (accuracy 86.8%, PPV 87.0%, NPV 86.7%). A summary of the diagnostic efficacy of each tested marker and comparison to imaging accuracy in our current study is presented in Table 2.

Correlation between the studied parameters and the severity of appendicitis as recorded in operative and pathology reports was analyzed using Spearman's correlation test. All of the parameters that were identified as having a significant diagnostic value were also found to significantly correlate with disease severity, including total bilirubin. ALT levels significantly correlated to pathological findings ($P = 0.023$) and had a trend to correlate to operative findings, which did not reached statistical significance ($P = 0.09$). MultiVar, sonographic graded severity, and CT graded severity were found to have the highest significant correlation to operative (Rho's coefficient 0.737, 0.703, and 0.691, respectively, $P < 0.01$) and pathological findings (Rho coefficient 0.662, 0.662, and 0.734, respectively, $P < 0.01$).

Prognostic significance was further assessed by correlation between the studied parameters and the LOS using Spearman's correlation test. All of the parameters with significant diagnostic value were found also to significantly correlate to length of stay. Sonographic graded severity, MultiVar, and CT graded severity were found to have the highest correlation with length of stay (Rho's coefficient 0.534, 0.570, and 0.673, respectively, $P < 0.01$).

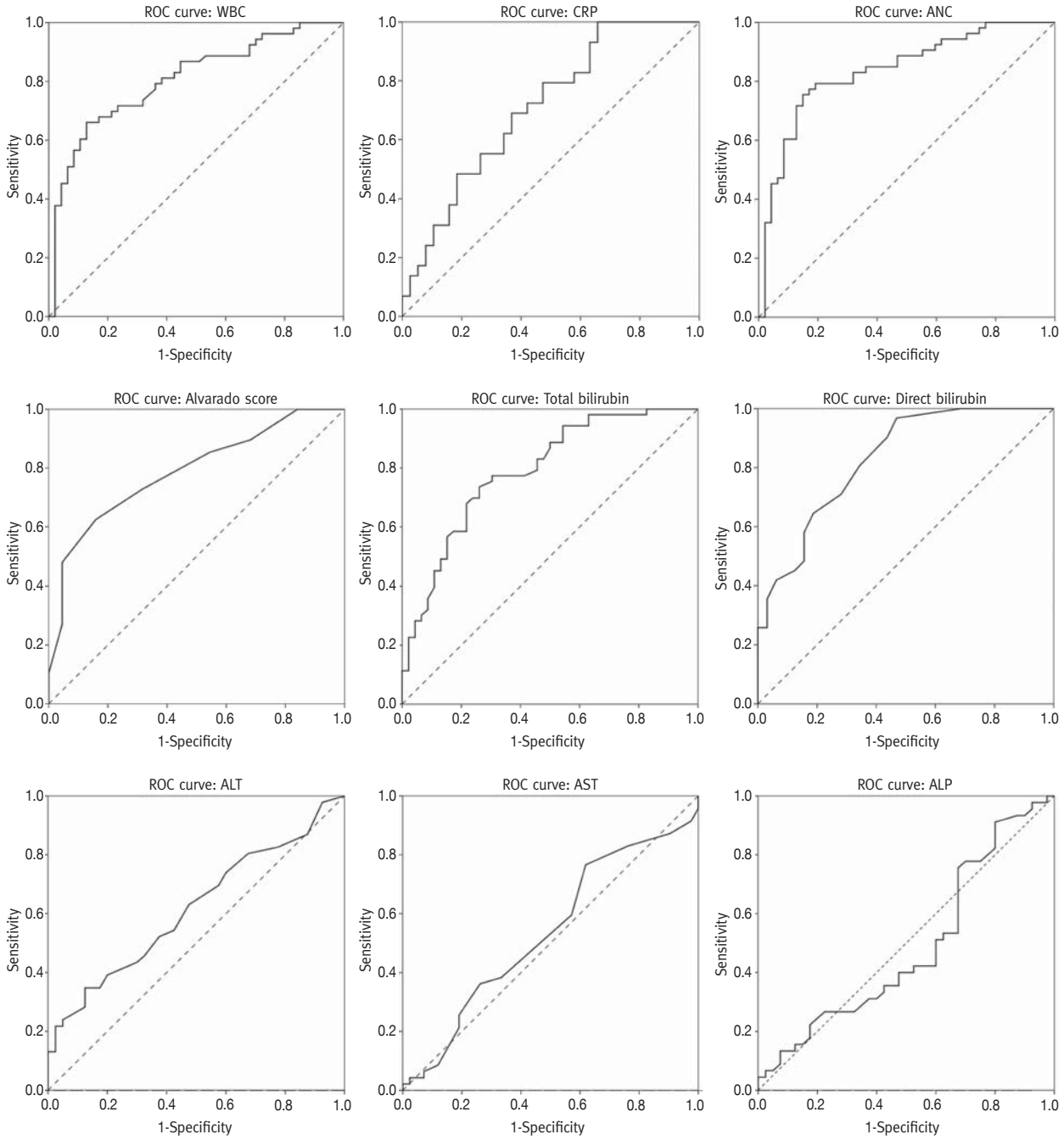
DISCUSSION

Diagnosis of acute appendicitis largely remains a clinical diagnosis supported by laboratory and imaging studies. Although several clinical scoring systems have been introduced, their accuracy remains moderate and similar to standard clinical judgment [8-10]. The use of modern imaging may significantly increase diagnostic accuracy, but may be limited by availability, cost, and radiation exposure. Difficulty in the diagnosis of acute appendicitis has led to the continuous search for better diagnostic markers [11,12] that may decrease radiation exposure and reduce costs.

KEY RESULTS

Our study assessed the diagnostic efficacy of bilirubin and other related markers in acute appendicitis. Similarly to the findings of D'Souza and colleagues [13], our results suggest that bilirubin levels may serve as an important diagnostic factor similar to commonly known factors such as WBC, CRP, and Alvarado score. Moreover, the combination of the ANC, serum bilirubin, and alanine transaminase levels have yielded accuracy similar

Figure 1. Receiver operating characteristic (ROC) analysis for diagnostic accuracy of acute appendicitis markers



WBC = white blood cell count, CRP = C-reactive protein, ANC = absolute neutrophils count, AST = aspartate transaminase, ALT = alanine transaminase, ALP = alkaline phospatase

to abdominal sonography (86.8% and 94.1%, respectively) and higher positive likelihood ratio compared to Alvarado scoring and imaging tests. In addition, correlation analysis of the markers showed bilirubin and the MultiVar combination to be among the top significantly correlated parameters to the disease severity. Such findings, if corroborated by further studies, may assist in decision making on operative management of complex clinical cases. In our study, the vast majority of acute appen-

dicitis cases underwent laparoscopic appendectomy using the same institutional protocol of operative and postoperative care. Analysis of the length of stay revealed that bilirubin, the MultiVar combination, and imaging findings were also found to significantly correlate with the length of stay, emphasizing the clinical prognostic relevance of these parameters.

LIMITATIONS

Our study was designed to assess whether serum markers could be used as a supporting tool for an emergency department in the diagnosis and management of patients suspected of having acute appendicitis. Although our results suggest that elevated bilirubin and liver enzymes are strong factors supporting the diagnosis of acute appendicitis, there are some limitations that warrant discussion. Our study is limited by the lack of follow-up on patients who were discharged from the ER. However, 75% of the patients participating in the study underwent at least one imaging study and only nine patients were discharged from the ER based on clinical evaluation alone. Another possible bias is that patients with abdominal pain from other reasons, such as urinary tract infection or pelvic inflammatory disease, may not have been referred to surgical assessment. Similarly, it is possible that patients with atypical presentation of acute appendicitis may have been solely treated by the ER physicians without arriving at the diagnosis of appendicitis and therefore were not referred for a surgical consult at the ER. The diagnosis on discharge for patients without acute appendicitis was not recorded in this study and therefore other causes of abdominal sepsis could not be sub-analyzed to determine their impact on bilirubin and liver enzyme levels.

INTERPRETATION

Over the past 5 years, several studies have suggested serum bilirubin and certain liver enzymes, such as AST and ALT, as possible diagnostic markers for acute appendicitis [Table 3] [1,2,13-15]. An even larger group of studies has focused on the association of serum bilirubin levels to the severity of appendicitis and appendiceal perforation. A large meta-analysis [16] showed high specificity (82%) and a diagnostic odds ratio of 4.42 (95%CI 2.21–8.83) for elevated serum bilirubin levels (greater than 1 mg/dl or > 20.5 μmol/l) in diagnosing perforated appendicitis.

Although the association between elevated bilirubin levels and severe appendiceal infections were described by Miller and Irvine more than half a century ago, the mechanisms leading to the observed elevation in serum bilirubin and liver enzymes are yet not fully understood. Jaundice and elevated liver enzyme levels have been well documented in patients with sepsis. The two primary pathogens isolated in acute appendicitis are *Bacteroides fragilis* and *Escherichia coli* (E. coli) [17], which cause endotoxemia, which is commonly related to sepsis-induced hepatic dysfunction. Exposure to E. Coli lipopolysaccharides (LPS) results in an inflammatory cascade [18], which down regulates bile related transporters, decreases hepatic metabolism [19-21], and increases nitric oxide synthase (iNOS)-dependent NO production, promoting hepatobiliary epithelial barrier dysfunction [22]. Furthermore, both bacterial species have been shown to affect with hepatocyte microcirculation, inducing sinusoidal damage in animal models [23].

Several high quality studies were published advocating non-operative management for acute appendicitis in specific settings [24]. In the Non-Operative Treatment for Acute Appendicitis (NOTA) study [25], the short-term success rate of antibiotic treatment in suspected appendicitis was 88% with no major adverse events recorded for patients with initial treatment failures. However, interpretation of these results needs to consider possible bias from the patient population with relative paucity of severe presentations (mean AIR score = 4.9, mean Alvarado score = 5.2). The findings in our study suggest that elevated serum bilirubin and ALT levels, while not purely specific for acute appendicitis, may indeed serve as markers to help in decision making and as such, possibly allow better selection of the proper treatment regimen.

CONCLUSIONS

The results of this study demonstrate that elevated levels of serum bilirubin and ALT may be considered as markers for the diagnosis of acute appendicitis as well as prognostic markers correlating with the severity of appendicitis. Combining serum bilirubin with ALT and ANC results in a marker that highly correlates with the diagnosis and prognosis of patients with acute appendicitis. This finding may be of special importance in pediatric and pregnant patients, due to the potential risk of CT scans or false diagnosis. Further population specific studies are needed to validate these findings in those groups.

Table 3. Diagnostic accuracy of acute appendicitis markers

	Source		Accuracy				
	Type	Group	WBC	CRP	Bilirubin	AST	ALT
Panagiotopoulou et al. [1]	Retro	N=1169 appendectomies	75%	67%	56%	NA	NA
Farooqui et al. [2]	Retro	N=1008 appendectomies	67%	66%	65%	52%	63%
D'Souza et al. [13]	Pros	N=242 cases of RLQ pain	70%	71%	65%	NA	NA
Emmanuel et al. [14]	Retro	N=472 appendectomies	78%	65%	42%	NA	NA
Al-Abed et al. [15]	Retro	N=447 appendectomies	71%	74%	51%	NA	NA
Current Study	Pros	N=100 cases of RLQ pain	69%	67%	70%	58%	57%

WBC = white blood cell count, CRP = C-reactive protein, AST = aspartate transaminase, ALT = alanine transaminase, Retro = retrospective, Pros = prospective, RLQ = right lower quadrant abdominal

Correspondence
Dr. A. Nevler
Dept. of Surgery, Thomas Jefferson University, Philadelphia, PA 19107, USA
Phone: (1-267) 303-3566
email: avinoam.nevler@jefferson.edu

References

1. Panagiotopoulou IG, Parashar D, Lin R, et al. The diagnostic value of white cell count, C-reactive protein and bilirubin in acute appendicitis and its complications. *Ann R Coll Surg Engl* 2013; 95 (3): 215-21.
2. Farooqui W, Pommergaard HC, Burcharth J, Eriksen JR. The diagnostic value of a panel of serological markers in acute appendicitis. *Scand J Surg* 2015; 104 (2): 72-8.
3. Parks NA, Schroeppel TJ. Update on imaging for acute appendicitis. *Surg Clin North Am* 2011; 91 (1): 141-54.
4. Alvarado A. A practical score for the early diagnosis of acute appendicitis. *Ann Emerg Med* 1986; 15 (5): 557-64.
5. Gomes CA, Nunes TA, Fonseca Chebli JM, Junior CS, Gomes CC. Laparoscopy grading system of acute appendicitis: new insight for future trials. *Surg Laparosc Endosc Percutan Tech* 2012; 22 (5): 463-6.
6. Guzmán-Valdivia Gómez G. An useful classification for acute appendicitis *Rev Gastroenterol Mex* 2003; 68 (4): 261-5. [Spanish]
7. Fallon SC, Kim ME, Hallmark CA, et al. Correlating surgical and pathological diagnoses in pediatric appendicitis. *J Pediatr Surg* 2015; 50 (4): 638-41.
8. Mán E, Simonka Z, Varga A, Rárosi F, Lázár G. Impact of the Alvarado score on the diagnosis of acute appendicitis: comparing clinical judgment, Alvarado score, and a new modified score in suspected appendicitis: a prospective, randomized clinical trial. *Surg Endosc* 2014; 28 (8): 2398-405.
9. Lintula H, Kokki H, Pulkkinen J, Kettunen R, Gröhn O, Eskelinen M. Diagnostic score in acute appendicitis. Validation of a diagnostic score (Lintula score) for adults with suspected appendicitis. *Langenbecks Arch Surg* 2010; 395 (5): 495-500.
10. Kollár D, McCartan DP, Bourke M, Cross KS, Dowdall J. Predicting acute appendicitis? A comparison of the Alvarado score, the Appendicitis Inflammatory Response score and clinical assessment. *World J Surg* 2015; 39 (1): 104-9.
11. Berger Y, Nevler A, Shwaartz C, et al. Elevations of serum CA-125 predict severity of acute appendicitis in males. *ANZ J Surg* 2016; 86 (4): 260-3.
12. Schellekens DH, Hulsewé KW, van Acker BA, et al. Evaluation of the diagnostic accuracy of plasma markers for early diagnosis in patients suspected for acute appendicitis. *Acad Emerg Med* 2013; 20 (7): 703-10.
13. D'Souza N, Karim D, Suntharesswaran R. Bilirubin: a diagnostic marker for appendicitis. *Int J Surg* 2013; 11 (10): 1114-7.
14. Emmanuel A, Murchan P, Wilson I, Balfe P. The value of hyperbilirubinaemia in the diagnosis of acute appendicitis. *Ann R Coll Surg Engl* 2011; 93 (3): 213-7.
15. Al-Abed YA, Alobaid N, Myint F. Diagnostic markers in acute appendicitis. *Am J Surg* 2015; 209 (6): 1043-7.
16. Giordano S, Pääkkönen M, Salminen P, Grönroos JM. Elevated serum bilirubin in assessing the likelihood of perforation in acute appendicitis: a diagnostic meta-analysis. *Int J Surg* 2013; 11 (9): 795-800.
17. Bennion RS, Baron EJ, Thompson JE Jr, et al. The bacteriology of gangrenous and perforated appendicitis--revisited. *Ann Surg* 1990; 211 (2): 165-71.
18. Geier A, Fickert P, Trauner M. Mechanisms of disease: mechanisms and clinical implications of cholestasis in sepsis. *Nat Clin Pract Gastroenterol Hepatol* 2006; 3 (10): 574-85.
19. Sonawane BR, Yaffe SJ. Gram-negative endotoxin administration decreases hepatic drug-metabolizing enzymes during development in rats. *Pediatr Res* 1980; 14 (8): 939-42.
20. Ogawa R, Morita T, Kunimoto F, Fujita T. Changes in hepatic lipoperoxide concentration in endotoxemic rats. *Circ Shock* 1982; 9 (4): 369-74.
21. McDougal WS, Heimburger S, Wilmore DW, Pruitt BA Jr. The effect of exogenous substrate on hepatic metabolism and membrane transport during endotoxemia. *Surgery* 1978; 84 (1): 55-61.
22. Han X, Fink MP, Uchiyama T, Yang R, Delude RL. Increased iNOS activity is essential for hepatic epithelial tight junction dysfunction in endotoxemic mice. *Am J Physiol Gastrointest Liver Physiol* 2004; 286 (1): G126-36.
23. Rink RD, Kaelin CR, Giammara B, Fry DE. Effects of live Escherichia coli and Bacteroides fragilis on metabolism and hepatic pO2. *Circ Shock* 1981; 8 (5): 601-11.
24. Di Saverio S, Sibilio A, Giorgini E, et al. The NOTA Study: prospective study on the efficacy and safety of antibiotics (amoxicillin and clavulanic acid) for treating patients with right lower quadrant abdominal pain and long-term follow-up of conservatively treated suspected appendicitis. *Ann Surg* 2014; 260 (1): 109-17.
25. Varadhan KK, Neal KR, Lobo DN. Safety and efficacy of antibiotics compared with appendicectomy for treatment of uncomplicated acute appendicitis: meta-analysis of randomised controlled trials. *BMJ* 2012; 344: e2156.

Capsule

Irons in the fire

Although transplantation is a life-saving therapy, patients receiving new organs are at serious risk for invasive, potentially fatal infections. *Aspergillus fumigatus* is a particularly common and troublesome fungal pathogen, but its ability to invade transplant tissues is poorly understood. To evaluate this property, **Hsu** et al. infected transplants in mice. Bleeding caused by damage to small vessels in grafted airways led to increased tissue iron, a known growth factor for *Aspergillus*. Thus, therapies in development that block iron and protect blood vessels may extend the life of organ recipients.

Sci Transl Med 2018; 10: eaag2616

Eitan Israeli

Capsule

A trehalose tool for tuberculosis

Tuberculosis is the leading infectious killer worldwide. The prevalence of drug- and multidrug-resistant *Mycobacterium tuberculosis* necessitates more rapid and specific diagnostics. **Kamariza** and colleagues designed a color-changing dye based on trehalose, a sugar that makes up the outer membrane of *M. tuberculosis*. The dye stained the live bacteria within minutes, emitting fluorescence with incorporation into the hydrophobic mycobacterial membrane. Heat-inactivated bacteria did not fluoresce and drug-treated bacteria emitted reduced fluorescence. This trehalose-based dye does not require sample washing and emits minimal background fluorescence, potentially making it particularly useful for the rapid detection of metabolically active *M. tuberculosis* in resource-limited environments.

Sci Transl Med 2018; 10: eaam6310

Eitan Israeli