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

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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.

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 evaluation.

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. specificity 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.
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 reach 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...
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. 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 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, 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**

<table>
<thead>
<tr>
<th>Source</th>
<th>Type</th>
<th>Group</th>
<th>WBC Accuracy</th>
<th>CRP</th>
<th>Bilirubin</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panagiotopoulou et al.</td>
<td>Retro</td>
<td>N=1069 appendectomies</td>
<td>75%</td>
<td>67%</td>
<td>58%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Farosqui et al. [2]</td>
<td>Retro</td>
<td>N=1008 appendectomies</td>
<td>67%</td>
<td>66%</td>
<td>65%</td>
<td>52%</td>
<td>63%</td>
</tr>
<tr>
<td>D’Souza et al. [13]</td>
<td>Pros</td>
<td>N=242 cases of RLQ pain</td>
<td>70%</td>
<td>71%</td>
<td>65%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Emmanuel et al. [14]</td>
<td>Retro</td>
<td>N=472 appendectomies</td>
<td>78%</td>
<td>65%</td>
<td>42%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Al-Abed et al. [15]</td>
<td>Retro</td>
<td>N=447 appendectomies</td>
<td>71%</td>
<td>74%</td>
<td>51%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Current Study</td>
<td>Pros</td>
<td>N=100 cases of RLQ pain</td>
<td>68%</td>
<td>67%</td>
<td>70%</td>
<td>58%</td>
<td>57%</td>
</tr>
</tbody>
</table>

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

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References


Capsule

Iron 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 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.

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
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