

## Serum Biomarkers As a Diagnostic Aid in Acute Appendicitis

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### Abstract

**Background:** Acute appendicitis is one of the most common acute intra-abdominal infections seen in surgical departments, which can be treated easily if an accurate diagnosis is made on time. Otherwise, delay in diagnosis and treatment can lead to perforation of the appendix & diffuse peritonitis.

**Materials and Methods:** A prospective study was conducted on 112 patients who were operated for acute appendicitis in department of General surgery, Bowring & Lady Curzon Hospitals, BMCRI, to determine the role and predictive value of the total leucocyte count (TLC), C-reactive protein (CRP) and percentage of neutrophil count in the diagnosis of acute appendicitis. Preoperative TLC, CRP and percentage of neutrophil count were determined and were compared with the results of the histopathology of the removed appendix.

**Results:** Of all the patients studied, 94 had histopathologically positive appendicitis. The TLC was found to be significantly high in 82 patients who proved to have acute appendicitis, whereas CRP was high in 88 patients and neutrophil percentage was raised in 87 patients; 6 patients had a normal CRP level. Thus, TLC had a sensitivity, specificity & positive predictive value of 87.23%, 44.44% and 89.13% respectively. CRP had a sensitivity, specificity & positive predictive value of 93.61%, 77.77% and 95.65% respectively. Percentage of neutrophil count had a sensitivity, specificity & positive predictive value of 92.55%, 38.88%, 88.77% respectively. When used in combination, there was a marked improvement in the sensitivity, specificity and the positive predictive value to 98.76%, 86.66% and 97.56% respectively.

**Conclusion:** The inflammatory markers, i.e., TLC, CRP and neutrophil count can be helpful in the diagnosis when measured together as this increases their sensitivity, specificity & positive predictive value significantly.

**Keywords:** Acute appendicitis, C-reactive protein, neutrophil count, TLC

### 1. Introduction

General surgeons have been facing acute appendicitis from hundreds of years. Appendicitis is the most common acute surgical condition of the abdomen [1]. Approximately 1-2% of the population will have appendicitis in their lifetime with the peak incidence occurring between the age of 10 and 30 years [2]. Its accurate preoperative diagnosis still remains elusive. The overall negative laparotomy rate remains at about 15-30% [3]. In women of a childbearing age, this rate is nearly doubled because of the prevalence of gynecologic diseases, the figure being as high as 30-50% [4]. Among young male patients, the negative appendectomy rate is relatively low (5-22%) [5]. In young children, the diagnosis may be incorrect in 30-46% of the cases. The classical triad of a compatible history, pain at McBurney's point and leukocytosis has a diagnostic accuracy of < 80%. Along with these, if ultrasonography & computerized tomography are included, it hardly reaches 90% [6].

It must be stressed that acute appendicitis is essentially a clinical diagnosis. Yet, laboratory measurements such as leucocyte count and C-reactive protein concentration are commonly used as diagnostic aids in patients with right lower quadrant abdominal pain. A polymorphic leukocytosis is stressed as an important feature for diagnosing acute appendicitis. An elevated level of C-reactive protein (CRP), an acute phase protein, is one of many downstream indicators of inflammation. Physiologically, CRP enhances cell-mediated immunity by promoting phagocytosis, accelerating chemo-taxis, and activating platelets. The test for CRP is a simple and effective screening test for occult bacterial infection or tissue injury. The aim of this study is to investigate the role of these inflammatory markers in diagnosing acute appendicitis.

The purpose of this study was to study the preoperative leucocyte count, percentage of neutrophil count and CRP levels (triple test) in patients suspected of having

acute appendicitis and to evaluate the preoperative diagnostic assurance and the predictive value of these tests in patients with acute appendicitis who underwent appendectomy at a later stage.

All the three tests (triple test) are easily available blood tests, & are not very expensive and the definite advantage is that they can be obtained within about 1-2 h. Thus, the surgeon on call can decide about the management of patients suspected of acute appendicitis well in time before complications ensue.

## 2. Materials & methods

The study was conducted on patients with a clinical diagnosis of acute appendicitis, who underwent appendectomy at emergency OT. These data were collected between August 2015 to June 2016. A total of 112 patients who were included in the study were operated for acute appendicitis at the Department of General Surgery, Bowring & Lady Curzon Hospitals, BMC&RI, Bengaluru. Only those patients who presented within 3-4 days of onset of symptoms with clinical diagnosis of acute appendicitis were included in the study. Interval appendectomy cases were excluded from the study. Decision to operate was not influenced by the preoperative levels of these tests. All patients were subjected to the following: (1) Clinical examination. (2) Routine blood tests immediately after decision to admit including full blood count and CRP. (3) Urine test. (4) Pregnancy test for all females. (5) Ultrasound scan to rule out other causes of abdominal pain.

A review of their preoperative total leucocyte count (TLC) and CRP levels and percentage of neutrophil count was made. The sensitivity, specificity, positive predictive value & diagnostic accuracy of these tests were calculated.

The cut-off value for white cell count was taken as 10,000/L. This value was selected arbitrarily as it corresponds to the elevated TLC. Percentage of neutrophil was considered elevated when >75%. The decision of operation was made independent of CRP level. The laboratory staffs were also not aware of the clinical findings, decision and the outcomes. Normal CRP level in our laboratory was < 6mg/dl and the level 6mg/dl and above was considered as raised/positive for this study.

All the patients were operated for appendicitis on the basis of history, physical findings and relevant clinical data. Postoperatively, the removed appendix was sent for histopathological examination. Based on histological features of the removed appendix, the patients were divided into three groups as follows: **Group A:** Normal appendix. **Group B:** Inflamed appendix (simple appendicitis). **Group C:** Perforated/gangrenous appendix (complicated appendicitis).

The number of patients with (1) both values normal, (2) only leucocyte count raised, (3) only CRP level raised and (4) both values raised were calculated in each of the three groups.

## 3. Results

A total of 112 patients were included in this study of whom 18 had a normal appendix histopathologically (Group A), giving an overall negative appendectomy rate of 16.07%. In this study, 68 (60.71%) patients were males and 44 (39.28%) were females, the male to female ratio being 1.5:1 [Table 1]. The age range was 11-75 years, with a mean age of 27.6 years [Table 2].

Among the 94 patients who had appendicitis, 66 had an inflamed appendix (Group B, simple appendicitis) and 28 had a ruptured/perforated/gangrenous appendix (Group C, complicated appendicitis) [Table 9].

The TLC was elevated in 82 patients and CRP was elevated in 88 cases, among the patients with positive histopathology (Group B + C). 6 patients had normal CRP and 12 patients had normal TLC. Of the 18 patients with negative appendix, 14 patients had a normal CRP level and only 8 patients had normal TLC [Table 5&6]. Again, in patients of 94 (Groups B + C), 78 had both TLC and CRP value raised and 6 patients had one of the either values in the normal range. Of the 18 patients with negative appendix, 2 patients had both TLC and CRP values raised, 12 patients had both values in the normal range and the remaining 4 patients had either of the values raised [Table 7].

When all the three parameters were combined (TLC, CRP and percentage of neutrophil count), of the 94 patients positive for appendicitis (Groups B and C), 80 patients had all the three values raised and only 1 patient had all the three values in the normal range & 13 patients had one or more values in the normal range. Among Group A, only 2 patients had all the three values raised and 16 patients had one or more values in the normal range & 13 patients had all the three values in the normal range [Table 8 & 9].

The sensitivity & specificity of TLC in this study were 87.23% & 44.44% and that for CRP were 93.61% & 77.77%, respectively. The positive predictive values for TLC and CRP were 89.13% and 95.65%, respectively ( $P < 0.001$ ). The combined TLC and CRP had a sensitivity, specificity and positive predictive value of 97.5%, 85.71% and 97.5%, respectively. When all the three parameters (TLC, CRP and percentage of neutrophil count) were combined, the specificity was increased to 86.66% and the positive predictive value improved to 97.56% [Table 10].

**Table 1: Percentage of appendectomy**

Sex	No of patients	Percentage
Males	68	60.71%
Females	44	39.29%
<b>Total</b>	<b>112</b>	<b>100%</b>

**Table 2: Percentage of appendectomy**

Age group in years	Number of patients	Percentage
11-20	25	22.32%
21-30	51	45.53%
31-40	29	25.89%
41-50	04	3.57%
51-60	Nil	Nil
>61	03	2.67%

**Table 3: Clinical Symptoms**

Clinical Symptoms	No of patients	Percentage
Right lower abdomen pain	112	100%
Nausea	108	96.43%
Vomiting	80	71.42%
Fever	32	28.57%

**Table 4: USG Diagnosis**

USG Diagnosis	No of patients	Percentage
Normal appendix/not visualized/probe tenderness (<6mm)	32	28.57%
F/S/O Acute appendicitis (diameter >6mm)	80	71.43%
<b>Total</b>	<b>112</b>	<b>100%</b>

**Table 5: TLC**

TLC	Group A	Group B	Group C	Total
Raised	10	58	24	92
Normal	08	08	04	20
<b>Total</b>	<b>18</b>	<b>66</b>	<b>28</b>	<b>112</b>

**Table 6: CRP**

CRP	Group A	Group B	Group C	Total
Raised/positive	04	62	26	92
Normal/negative	14	04	02	20
<b>Total</b>	<b>18</b>	<b>66</b>	<b>28</b>	<b>112</b>

**Table 7: TLC & CRP**

TLC & CRP	Group A	Group B	Group C
Both raised	02	55	23
Both normal	12	02	Nil
<b>Total</b>	<b>14</b>	<b>57</b>	<b>23</b>

**Table 8: TLC+CRP+Neutrophil percentage**

TLC+CRP+Neutrophil percentage	Histologically proven appendicitis (Group B+C)	Histologically normal appendix (Group A)	Total
All raised	80	02	82
All normal	01	13	14
<b>Total</b>	<b>81</b>	<b>15</b>	<b>96</b>

**Table 9: TLC, CRP and percentage of neutrophil count**

Group	Operative findings	No of patients	TLC raised	CRP raised	% of neutrophil count raised	TLC & CRP raised	All raised
A	Uninflamed appendix	18	10	04	11	02	02
B	Inflamed but uncomplicated appendix	66	58	62	61	55	54
C	Complicated appendix	28	24	26	26	23	26

TLC, CRP and percentage of neutrophil count

**Table 10:**

Tests	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
<b>TLC</b>	87.23%	44.44%	89.13%	40%	80.35%
<b>CRP</b>	93.61%	77.77%	95.65%	70%	91.07%
<b>TLC+CRP</b>	97.5%	85.71%	97.5%	85.71%	95.74%
<b>TLC+CRP+% Neutrophils</b>	98.76%	86.66%	97.56%	92.85%	97.89%

#### 4. Discussion

Acute appendicitis is the most common cause of emergency abdominal surgery [7]. Because clinical diagnosis of acute appendicitis is difficult, appendectomy after false-positive diagnosis of appendicitis (hereafter, negative appendectomy) is performed in up to 15-25% of the cases [8]. Some authors have even reported negative appendectomy rates of up to 50% in women of the reproductive age group [9]. Such negative explorations have been accepted as an unavoidable consequence of the principle of early exploration to prevent perforation of the appendix, but this practice is being questioned increasingly.

A majority of the patients with acute appendicitis present with right-sided lower abdominal pain, nausea and vomiting, but these symptoms are very nonspecific. In fact, any acute abdominal condition can mimic appendicitis and thus the list of differential diagnosis is long and hence removal of a normal appendix is not unusual. Localized tenderness and evidence of peritoneal inflammation (guarding and rebound tenderness) make the diagnosis probable. Laboratory investigations usually contribute little and can be misleading [10].

Although appendectomy is considered to be a safe operation, it has still got associated complications, most noticeable among them being wound infection, intra-abdominal abscess, adhesions and bowel obstruction and pulmonary complications from general anesthesia. Additionally, some patients have persistent symptoms even after the surgery. Such patients constitute as a burden on the hospital resources while being generally unsatisfied with the health care providers.

Appendectomy for a normal appendix is associated with both mortality and morbidity [11]. Some reports indicate a higher risk for intestinal obstruction following surgery for a normal appendix compared with that for a non-perforated inflamed appendix [12,13]. The risk for intestinal obstruction is increased by up to 5% in patients with a healthy appendix [14].

Ultrasonography (US) is appropriate in patients in whom the diagnosis is equivocal by history and physical examination. It is especially well suited in evaluating right lower quadrant pain in pediatric and female patient. Computed Tomography (CT) scan is more helpful in evaluating patient with suspected appendicitis [15]. The negative appendectomy rate fall from 20.0% before the use of

CT to 7.0% after its introduction [16]. Laparoscopy an invasive modality may be helpful in equivocal cases or in women of childbearing age group. It can be both diagnostic and therapeutic. But the rate of removal of normal appendix is also high with laparoscopy [17].

Raised levels of Total leukocyte count (TLC) with clinical signs and symptoms point towards acute appendicitis but patients with perforated appendix can still have normal TLC and differential leukocyte count. The white value of blood cell count is elevated (greater than 10,000/mm<sup>3</sup>) in 80 percent of all cases of acute appendicitis [18]. Unfortunately, the WBC is elevated in up to 70 percent of patients with other cause of right lower quadrant pain. Thus a serial elevated WBC measurement (over 4-8 hours) in suspected cases may increase the specificity, as the WBC count often increases in acute appendicitis 10 (except in case of perforation, in which it may initially fall)[19].

**Table 11: Sensitivity and specificity of white blood cell count in diagnosis of acute appendicitis**

References	Sensitivity	Specificity
Yu et al[20]	62	75
Yang et al[21]	86	32
Xharra et al[22]	85	68
Wu et al[23]	80	71
Agrawal et al[24]	79	55
Mentes et al[25]	72	77

C reactive protein is the prototype acute phase reactant, synthesized by liver, its concentration rises within 8 hours of onset of tissue injury/inflammation, peaks 24 – 48 hours and remains elevated as long as continuing tissue inflammation or destruction. CRP was identified in 1930 by Tillett and Francis as a substance in the serum of patients with acute inflammation that reacted with the C polysaccharide of pneumococcus. Initially it was thought that CRP might be a pathogenic secretion as it was elevated in people with a variety of illnesses including cancer, however discovery of hepatic synthesis demonstrated that it is a native protein [26]. The CRP gene is located on the first chromosome (1q21-q23). CRP is a 224-residue protein with a monomer molar mass of 25106 Da. The protein is an annular pentameric disc in shape and a member of the small pentraxins family [27].

There have been many reports emphasizing the value of CRP in improving the diagnostic accuracy of acute appendicitis. Mustard et al. documented that serial postoperative CRP levels could predict septic complications before their clinical manifestation [28]. Multivariate analysis by Oosterhuis et al showed that serial CRP measurement could improve the accuracy of diagnosing acute appendicitis [29]. In addition, a recent (1997) meta-analysis of 22 published articles concluded that CRP is a test of medium accuracy in diagnosing acute appendicitis [30]. Diagnostic accuracy of serum CRP of different study is shown in the Table-12. Diagnostic accuracy of CRP in our study was

91.07%. Regular measurement of TLC and CRP in suspected appendicitis may improve accuracy of diagnosing acute appendicitis.

**Table-12: Diagnostic efficacy of serum C-reactive protein**

References	Sensitivity	Specificity	Cut of value CRP (6mg/dl)
Nordback and Harju[31]	48.5	84.4	-
Sodena et al[32]	58	72	>10mg/dl
Al-Saigh[33]	39.7	76.3	-
Oosterhuis et al[29]	87	50	>6mg/dl
Ko et al[34]	51	95	>5mg/dl
Gurleyik et al[35]	93.5	80	-
Asfar[36]	93.6	86.6	-
Agrawal et al[24]	74.8	66.7	>6mg/dl

The aim of our study was to determine the diagnostic accuracy of TLC, CRP and neutrophil count in combination in the diagnosis of acute appendicitis. Our study concluded that based on unaided clinical signs and symptoms, diagnostic accuracy of acute appendicitis was less than 80%. The sensitivity and specificity of TLC, CRP and percentage of neutrophil in the diagnosis of acute appendicitis was calculated individually and in combination. It was observed that when combined, the specificity and positive predictive value were raised significantly, with a greatly improved probability of diagnosing acute appendicitis in equivocal cases.

### 5. Conclusion

We concluded that if patients with right iliac fossa pain were explored on the basis of preoperative serum CRP levels and TLC counts, and due respect was given to the percentage of neutrophil count, 8 out of 18 negative explorations would have been prevented thus preventing the morbidity and burden on hospital resources associated with these negative explorations. If just positive serum CRP would have taken into account 14 out of 18 negative appendectomy would have been prevented. Therefore, we recommend performing all three of these laboratory tests in combination in patients with an equivocal diagnosis of acute appendicitis based on clinical signs alone, before surgical exploration.

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