

Clinical Accuracy of Inflammatory Markers Combined with Ultrasound in Acute Appendicitis

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ABSTRACT

Objective: To determine the clinical accuracy of inflammatory markers combined with ultrasound, in acute appendicitis keeping histopathology as the gold standard.

Study design: Cross Sectional (validation) study.

Study setting: Surgical B ward, Department of Surgery Khyber teaching hospital Peshawar.

Duration of Study: 07 months i.e. from 1st September 2014 to 31st of Mar, 2015

Methodology: All patients having acute appendicitis were subjected to blood sampling for CRP, TLC and neutrophil%. After that, all these patients were subjected to ultrasonography to detect the presence or absence of appendicitis. All removed appendices were sent for histopathology which was taken as the gold standard.

Results In the present study, TLC had the highest sensitivity (77.68%) followed by neutrophil% (69.96%), CRP (67.10%) and U/Sound (62.96%) respectively. While U/Sound had the highest specificity (70.59%) followed by CRP and TLC (64.71% each) and neutrophil% (58.82%) respectively. When all the four tests were combined the sensitivity, specificity, (99.17% and 98.45%) increased significantly. It was seen that when all the four tests were negative, appendicitis could be safely ruled out and surgery could be deferred in these patients. It would reduce the rate of negative appendicectomies.

Conclusion: TLC contains important diagnostic information and hence should always be included in the diagnostic workup of acute appendicitis. The sensitivity of CRP and Neut. % is low individually, but when combined with TLC and u/sound the sensitivity and specificity increases significantly. But it is stressed that history and clinical examination by a skilled surgeon still remain important in diagnosing acute appendicitis, and its importance cannot be denied. The investigations can be used as an adjuvant to a surgeon's clinical diagnosis.

Keywords: Acute Appendicitis; CRP; TLC; Neutrophil percentage; Ultrasound; Histopathological report

Introduction

The vermiform appendix is considered to be a vestigial organ by most, its importance in surgery results only from its tendency for inflammation that results in a clinical syndrome known as acute appendicitis and is the most common indication for emergency abdominal surgery in patients with acute abdomen. In general, there is a crude annual incidence of 26 per 10,000 population.^{1,2}

Traditionally acute appendicitis has been a clinical diagnosis based on patients history and physical examination,^{1,3} but the

accuracy of clinical diagnosis ranges from 70-85%.^{2,4} Therefore histopathology remains the gold standard for confirming acute appendicitis.⁵ In an age accustomed to early and accurate preoperative diagnosis acute appendicitis remains an enigmatic challenge,¹ as it is complicated by nonsurgical diseases that mimic appendicitis,³ and a decision to operate based on clinical suspicion alone can lead to the removal of a normal appendix in 15-30% of cases i.e. negative appendectomies.^{1,4,6} Such patients constitute as a burden on the health care system and improving

the diagnostic protocol may help in decreasing the rate of negative appendectomies.⁴⁻⁷

Although appendectomy is considered to be a safe operation it still has got associated complications; morbidity of 3.1% in acute appendicitis and up to 47.2% in cases where perforation has occurred.^{7,8} The accuracy of diagnosis can be enhanced by investigatory modalities such as ultrasonography, CT scan, magnetic resonance imaging and inflammatory markers which are characterized as noninvasive, understandable and cost-effective,^{4,9,10} but of the various commonly used diagnostic aids for appendicitis no single test alone can reduce the rate of a negative appendectomy to zero.^{2,11} Hence some authors have recommended a combination of two or more investigations to increase accuracy more.^{2,12-14} and the use of noninvasive modalities such as the use of ultrasonography and common blood inflammatory markers is gaining support.^{3,12-17}

Addition of routine ultrasonography in clinical assessment for acute appendicitis can improve the diagnostic accuracy with sensitivity ranging from 75-98%, specificity of 73-100% positive predicted value of 84-100% and negative predicted value of 89-96%.^{2,3,6,12} even though some institutions have reported contradictory results,³ mainly because ultrasound is operator dependent and need significant experience to identify positive findings.^{5,10} but the additional use of ultrasonography can also prove helpful in ruling out other pathologies mimicking acute appendicitis and thereby reduce the rate of negative appendectomies.^{8,12,15}

C reactive protein is synthesized by hepatocytes during the acute response phase to a variety of infections or inflammatory disease processes. The reported predicted values of c reactive protein in appendicitis vary widely with sensitivity ranging from 40-99% and specificity from 27-90%.^{17,18} a lot of authors conclude that a normal c reactive protein value probably indicates a normal noninflamed appendix.^{17,18-19} Some investigators have stressed the importance of these inflammatory blood markers in the context of deciding upon discharge or admission for further investigations/surgery.^{14,17} many studies suggest that TLC and CRP, when combined, can significantly increase the diagnostic accuracy. The overall sensitivity increases ranging from 83-100%, specificity of 88-92% and positive predictive value of 95-98%.^{7,13,14,18}

Very few studies have been done on evaluation of the diagnostic accuracy of inflammatory markers in combination with imaging studies in acute appendicitis. Though a diagnostic pathway using ultrasonography and clinical re-evaluation for patients with acute abdomen provided excellent results for the

diagnosis and treatment of appendicitis in a study carried by Boudewijn R et al.³ moreover in another study Gülten K et al suggested that TLC with the help of ultrasonography could be helpful in the diagnosis of appendicitis especially in females during the reproductive period.²¹ while a study by s.ali et al. Suggested that combining u/sound, CRP, TLC and neutrophil% increased the specificity markedly.²²

However, to the best of my knowledge, no local study has been performed to evaluate the significance of u/sound in combination with TLC, neutrophil% and CRP in the diagnosis of acute appendicitis. The rationale behind this study is to determine the diagnostic accuracy of u/sound in combination with TLC, neutrophil% and CRP in acute appendicitis while keeping histopathology as the gold standard in clinically diagnosed cases of acute appendicitis. The idea behind my study is that a combination of these easily available and cheap tests can improve the diagnostic accuracy from mere clinical evaluation or a single diagnostic test and hence reduce the rate of negative appendectomies where ever possible. In my study, if the sensitivities and specificities are found to be significantly high or equal to studies available in literature than it can be suggested that a new protocol for improved management of acute appendicitis be developed to reduce the rate of negative appendectomies and this may be useful in reducing the burden on health care system.

Methodology

The study was a cross sectional validation study conducted in Surgical B-ward, Department of Surgery, Khyber Teaching Hospital, Peshawar. The study was conducted after approval from hospital's ethical and research committee. The duration of the study was 07 months i.e. 1st September 2014 to 31st Mar, 2015 and non-probability consecutive sampling was taken as a sampling technique for the purpose of patient's data collection. All Patients both male and female admitted through emergency with highly suspected clinical features of acute appendicitis (i.e. tender right iliac fossa, migratory pain, rebound tenderness, anorexia, nausea or vomiting) with age more than 15 years and less than 60 years were taken as inclusion criteria of the study. Exclusion criteria includes patients with history of previous abdominal surgery or recent trauma, patients with history of renal stones or chronic liver disease (HBs HCV) and or other inflammatory diseases or having a palpable mass in RIF, female patients with history of amenorrhea or pregnancy or other gynecological pathology found incidentally on ultrasonography, patients diagnosed with

any other pathology on u/sound, or having clear signs of generalized peritonitis.

Appendicitis on histology included all grades of the inflamed appendix, having histologically proven transmural acute inflammatory changes with or without signs of localized collection. TLC and Neutrophil% was considered positive if value of TLC more than 9600/mm³ and Neutrophilia of more than 70% shall be considered as positive. Positive Ultrasound of appendix identified when tender RIF and non-compressible or blind ended loop measuring minimum of 6mm in diameter or appendiceal phlegmon or abscess/collection of free fluid seen in RIF.

All patients were examined with a detailed history. All patients were subjected to blood sampling for CRP, TLC and neutrophil%. After that all patients were subjected to ultrasonography to detect the presence or absence of appendicitis. All removed appendices were fixed in 4% formalin and sent for histopathology to the hospital lab. Where they were analyzed by a histopathologist having a minimum of five years of experience for confirmation of inflammation. All laboratory tests were performed by the same lab under similar technique of sampling and testing. All ultrasonic procedures were done by the same consultant radiologist having a minimum of 5 years of experience. All the above-mentioned information including name, age, and gender were recorded in a pre-designed proforma. Strict exclusion criteria were followed to control confounders and bias in the study results.

SPSS version 20.0 was used for data collection and analysis. The study variables were the age, gender, Values of CRP, TLC, ultrasound findings and microscopic finding of Appendix. All results were presented as tables and charts. Sensitivity, Specificity, positive predictive value (PPV), negative predictive value (NPV) were determined for each test by taking histopathology reports as gold standard⁵ from 2x2 table. Sensitivity of CRP or TLC or Neutrophil% or U/sound = $(a / a+c) \times 100$. Specificity of CRP or TLC or Neutrophil% or U/sound = $(d / b + d) \times 100$. Positive predictive value (PPV) for CRP or TLC or Neutrophil% U/sound = $(a / a + b) \times 100$. Negative predictive value (NPV) for CRP or TLC or Neutrophil% or U/sound = $(d / c + d) \times 100$. Accuracy of CRP or TLC or Neutrophil% or U/sound = $(d + a) / \text{overall patients}$. Combined sensitivity and specificity were calculated using cross tabulation, and by using the statistical method of addition rule of probability that is used in such cases, taking all the four tests as independent of each event. Keeping in view the fact that a highly sensitive test if negative rules out the disease and highly specific test if positive rules in the disease.³⁰ Combined Specificity: $1 - (1 - \text{specificity of CRP}) \times (1 - \text{specificity of TLC}) \times$

$(1 - \text{specificity of Ultrasound}) \times (\text{specificity of neutrophil}\%)$. Combined Sensitivity: $1 - (1 - \text{sensitivity of CRP}) \times (1 - \text{sensitivity of TLC}) \times (1 - \text{sensitivity Ultrasound}) \times (\text{sensitivity of neutrophil}\%)$

Results

The study was performed on 250 patients who have been clinically diagnosed as cases of acute appendicitis and were admitted in the department of surgery KTH for an emergency appendectomy. The data was analyzed to seek the combined diagnostic accuracy of CRP total leukocyte count u/sound and neutrophils percentage in acute appendicitis by evaluating the sensitivity, specificity, positive and negative predictive values of each test individually and then calculating the combined sensitivity and specificity using statistical formulas. The results were analyzed with SPSS version 20.0.

The patients who presented with acute appendicitis have mean age of 24.93 ± 9.79 . In the study 162 (64.2%) out of 250 were male and 88 (35.2%) were female. There were 233 (93.2%) cases found to be positive on histopathology report and 17 (6.8%) were reported as negative on histopathology findings, as shown in Table I.

		Mean ± SD
Age (years)		24.93 ± 9.79
		n (%)
Gender	Male	162 (64.8)
	Female	88 (35.2)
Acute appendicitis*		233 (93.2)
Normal appendix		17 (6.8)

*Histopathology findings

Out of these 187 TLC positive patients 181 (72.4%) were true positive on the basis of histopathology report and 52 (20.8%) had a false negative test. The accuracy of TLC in this study was calculated as 76.8% also corresponding to the ROC curve analysis with sensitivity (77.68%) specificity (64.71 %) respectively. Out of 170 neutrophil% test positive patients, 163 (65.2%) were true positive on the basis of histopathology report and 70 (28.0%) had a false negative test. The accuracy of neutrophil% test in this study was calculated as 69.2% also corresponding to the ROC curve analysis with sensitivity (69.96%) specificity (58.82 %) respectively. Out of these 161 CRP test positive patients, 155 (62.0%) were true positive on the basis of histopathology report and 76 (30.4%) had a false negative test. The accuracy of CRP test in this study was calculated as 66.4% also corresponding to the ROC curve

analysis with sensitivity (67.10%) specificity (64.71 %) respectively. (Figure 1)

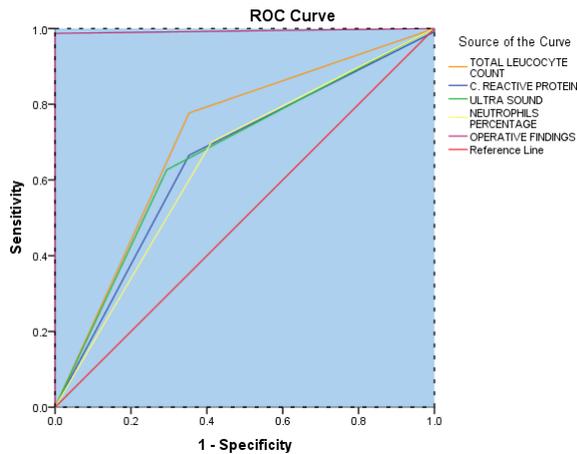


Figure 1. ROC for TLC, Neut%, CRP and USG

Out of these 151 u/sound test positive patients 146 (58.4%) were true positive on the basis of histopathology report and 87 (34.8%) had a false negative test. The accuracy of u/sound in this study was calculated as 63.2% also corresponding to the ROC curve analysis with sensitivity (62.66%) specificity (70.59%) as shown in Table. II and III.

Table II: Percentage of TLC, Neut%, CRP and USG with Histopathology findings

		Histopathology		Total
		Acute Appendicitis	Normal Appendix	
Gender	Male	156 (62.4)	6 (2.4)	162
	Female	77 (30.8)	11 (4.4)	88
TLC	positive	181 (72.4)	6 (2.4)	187
	negative	52 (20.8)	11 (4.4)	63
Neutrophils	positive	163 (65.2)	7 (2.8)	170
	negative	70 (28.0)	10 (4.0)	80
CRP	positive	155 (62.0)	6 (2.8)	161
	negative	76 (30.4)	11 (4.8)	87
Ultrasound	positive	146 (58.4)	5 (2.0)	151
	negative	87 (34.8)	12 (4.8)	99

Table III: Diagnostic Accuracy of TLC, Neut%, CRP and USG

	TLC	Neut%	CRP	USG
Sensitivity	77.68%	69.96%	67.10%	62.66%
Specificity	64.71%	58.82%	64.71%	70.59%
PPV	96.79%	95.88%	96.27%	96.69%
NPV	17.46%	12.50%	12.64%	12.12%
Disease Prevalence	93.20%	93.20%	93.15%	93.20%

Since, combined sensitivity and specificity is simply not an average of all tests neither could they be calculated using cross tabulation, the statistical method of addition or multiplication rule of probability that is used in such cases was employed. A

highly sensitive test if negative rules out the disease and highly specific test if positive rules in the disease.³¹

Table IV shows the combined sensitivity for TLC, CRP, neutrophils%, and ultrasound was 99.17% when the addition rule of probability was used but as a general rule when using the addition rule for sensitivity, specificity is calculated as the product of specificity of all tests which in this case became 17.38. When the addition rule of probability was used for combined specificity it was calculated to be 98.45%. Similarly, in this case as a general rule when using the addition rule for specificity, sensitivity is calculated as a product of sensitivity of all tests which was observed to be 22.78%. As far as tests, in general, are concerned it does not matter whether a rule in (high specificity) or rule out (high sensitivity) method is employed to make a diagnosis of appendicitis in an individual patient³⁰, either way, the diagnostic accuracy will be improved. In multiple testing situation, PPV and NPV are calculated for each test individually and not for the combined tests.³¹

Table No. 04 Combine statistics of TLC, CRP, Neut% and USG

Combined Sensitivity*	
TLC+CRP	92.63%
TLC+CRP+NEUT%	77.82%
TLC+CRP+NEUT%+USG	99.17%
Combined Specificity**	
TLC x CRP	41.80%
TLC x CRP x NEUT%	29.50%
TLC x CRP x NEUT% x USG	17.38%
Combined Sensitivity*	
TLC+CRP	58.20%
TLC+CRP+NEUT%	82.80%
TLC+CRP+NEUT%+USG	98.45%
Combined Specificity**	
TLC x CRP	52%
TLC x CRP x NEUT%	32.59%
TLC x CRP x NEUT% x USG	22.78%

Discussion

The study was performed in Department of General Surgery KTH from 1st September 2014 to 31st of March 2015. 250 patients were included in this study out of which 162 (64.2%) were male and 88(35.2%) were female. The male to female ratio shows a male predominance with a ratio of 1.84:1. The most common age group among both genders ranged from 15 to 25 years this finding co-relates to the literature which reports the peak incidence of acute appendicitis in the teens and early 20's.^{1,2}

Acute appendicitis is the most common abdominal surgical emergency requiring surgery¹ but due to lack of definitive preoperative tests the rate of negative appendectomy is still very high where according to literature, clinical accuracy ranges

from 70% to 95%^{2, 4, 6} which is based on experience and clinical skills of the surgeons. This puts a burden on the healthcare system because unnecessary surgery exerts a negative socio-economic impact in the form of hospital expenses and decreased productivity.⁴⁻⁷

In practice acute appendicitis is diagnosed clinically based on patient's history and examination by evaluating signs and symptoms like right iliac fossa pain, nausea, vomiting, low-grade fever and rebound tenderness in the right iliac fossa. In most cases there is a history of periumbilical pain later migrating to the right iliac fossa. But all these signs and symptoms are not specific to acute appendicitis leading to misdiagnosis in as much as up to 20% of the cases.^{1,2,3}

In the current study, clinical diagnosis was found to be correct in 233 (93.2%) patients while 17 (6.8%) were observed to be negative on histopathology. The rate of negative appendectomy in this current study is low but in concordance to the literature and might be attributed to the strict following of inclusion and exclusion criteria and thorough history and examination of the patients before labeling them as cases of acute appendicitis. Out of the total negative cases (as per histopathology report) it was observed that 11 (4.4% of total patients) were female and 6 (2.4% of total patients) were male. These results are supported by other studies as well and in a general observation, negative appendectomy rate is higher in female patients partly because of so many other conditions that might mimic acute appendicitis.³ Kemal M. Et al performed a study to evaluate the diagnostic value of preoperative laboratory and radiological studies for appendicitis. In their study, Negative appendectomy rate was 17.3% (27% for female, 11.5% for male). They concluded that besides the improvement of diagnostic tests for acute appendicitis, we could not sufficiently reduce the negative appendectomy rate.¹¹ Tariq W. Et al in their study reported an overall negative appendectomy rate of 12.3% and concluded that the preoperative ultrasonography is an essential tool for reduction of negative appendectomy rate.⁶ Similarly S Scammell et al and Mohammad A. A. M. Et al in their study reported the negative appendectomy rate as 16.5%¹² and 22.5% respectively.¹⁵ Raised TLC count is present in around three fourth of the patients with acute appendicitis but it might be raised in other acute inflammatory conditions as well.¹

In the current study 187 (74.8%) patients tested positive TLC test for acute appendicitis but after comparing with the gold standard (histopathology) 181(72.4%) were labeled as true positive. The sensitivity and specificity of TLC in this study was 77.68% and 64.71 % respectively while PPV and NPV was calculated as 96.79% and 17.46% respectively. Haider Kamran

et al in their study reported that TLC although not a diagnostic criterion for acute appendicitis but still is a helpful investigation in decision making.²⁰ Gülten Kiyak et al also suggested that if the leukocyte count is increased alone or with the help of USG evaluation could be helpful in the diagnosis of appendicitis especially in females during the reproductive period.^{21,24}

Our results have shown that TLC is more sensitive than CRP in the diagnosis of acute appendicitis. Our findings are consistent with the study of Khurram Siddique et al¹³ who concluded that Accuracy of TLC is higher than CRP for diagnosing acute appendicitis and also with Shehzad Ahmed Abbasi et al²⁴ who reported that TLC has more sensitivity and diagnostic accuracy in acute appendicitis in comparison to Ultrasound. In this study the p-value for TLC is 0.00 which is significant. Such a high positive predictive value (PPV) of TLC in cases of acute appendicitis suggests its high yield in the diagnosis of appendicitis.^{13,24} But in this study, it was found that TLC alone can't be used as sole diagnostic test. When TLC is combined with other tests like CRP, neutrophil percentage and ultrasound the sensitivity and specificity are significantly increased.^{9, 13, 14, 18, 20, 24,26} Arshad Kamal, et al suggested that the diagnostic value of Total leukocyte count is increased when it is combined with neutrophil count and C-reactive protein. When neutrophil count and total leukocyte count are combined only 4% patients with acute appendicitis will have normal values.²⁵

Moreover, it has been suggested in the literature that in equivocal cases the clinical diagnosis of acute appendicitis should be preferred over the TLC results. Usually, a shift to left is seen in the differential leukocyte count in most of the patients with acute appendicitis pertaining to increased neutrophils percentage. This finding also signifies an acute inflammatory response of the body to the diseased appendix.¹ most studies report that Neutrophilia of more than 75% is found in more than 75% of the cases.²⁰

In the current study, a total of 170 (68.0%) had a positive neutrophil% test. After comparing the results with the gold standard 163 (65.2%) were found the true positive. The sensitivity and specificity of neutrophil percentage in this study was 69.96% and 58.82 % respectively while PPV and NPV was calculated as 95.88% and 12.50% respectively. Adnan A. Mohammed et al wrote in their report that when there is an inflamed appendix there is an increase in the neutrophil response. Later on, when the invasion of bacteria occurs, there is a general increase in the total WBC count in addition to the pre-existing neutrophil response. Therefore, the diagnostic accuracy of WBC count can be improved if the neutrophil percentage is taken in consideration.²⁷

Rafael N. Goulart et al concluded that neutrophils% levels are related to the evolution stage of appendicitis.¹⁹ While S. Ali et al observed in their study that raised Neutrophil percentage was less sensitive (58.52%) and specific (61.90%) with higher mean values in gangrenous/perforated appendicitis. The sensitivity of raised Neutrophil percentage ranges from 60 to 84% in various studies and When TLC and Neutrophil percentage were combined the sensitivity improved to 87% .²²

The current study shows a fair association between acute appendicitis an Neut.% but as compared to TLC, it has got lesser sensitivity and specificity. Though the p value is significant at 0.05. When neutrophils percentage is combined with other tests like CRP, TLC and ultra sound the sensitivity and specificity are significantly increased.^{22, 7}

Sheikh Muzamil et al observed that when combined with other tests, the specificity and positive predictive value were raised, with a greatly improved probability of diagnosing acute appendicitis in equivocal cases⁷ similar results were observed in the current study. CRP is an acute phase reactant which is synthesized by the hepatocytes in acute/inflammatory disease processes. Its concentration increases within 8 to 12 hours of onset of acute infections/inflammations with peak values at 24-48 hours and remains high till the initiating process lasts. Many studies in the recent past have been done to evaluate its significance in acute appendicitis. The reported predicted values of C reactive protein in appendicitis vary widely with sensitivity ranging from 40-99% and specificity from 27-90%.^{17, 18} A lot of authors conclude that a normal C reactive protein value probably indicates a normal non inflamed appendix.^{17,18-19}

In the current study a total of 161 (64.4%) patients had a positive CRP test and out of these, 155 (62.0%) were true positive on the basis of histopathology report. The sensitivity and specificity of CRP in this study was 67.10% and 64.71 % respectively while PPV and NPV was calculated as 96.27% and 12.64% respectively. Ahmad Q.A., et al concluded that CRP is a better lab test than TLC in the diagnosis of complicated acute appendicitis. High levels of CRP either alone or with raised TLC required mandatory explorations and suggested that CRP should be done as routine laboratory test along with TLC in doubtful cases of acute appendicitis.¹⁴

Shyam S. Sahu et al in their study also reported that CRP had the highest sensitivity and specificity of all test that they performed in their study. (90%, 80%).²⁸ Shefki Xharra et al, Shozo Yokoyama et al and Adnan A. Mohammed et al reported that a raised value of the CRP was directly related to the severity of inflammation and that CRP is more accurate than

the WBC and neutrophil counts.^{18,27} As reported in other studies in this study it was observed that when CRP is combined with other tests like TLC neutrophil percentage and u/sound the sensitivity and specificity are significantly increased.^{18, 27, 29}

In a nutshell, it can be concluded that CRP cannot replace clinical diagnosis but it can be cost effective in the diagnosis of acute appendicitis. One limitation in this study regarding CRP is that a simple cut off value was used to label patient either positive or negative. Recent studies have reported an association between serum CRP values and the grade/severity of appendicitis.^{18,27} A lot of useful data could be obtained in the future by correlating the values of CRP with the severity of appendicitis. And hence a new protocol for the type of surgical approach and management could be formulated. The additional use of ultrasonography in clinical assessment has been shown to improve the diagnostic accuracy of acute appendicitis.^{2, 3, 6, 12} Ultrasound can also prove helpful in ruling out the pathologies mimicking acute appendicitis.^{8, 12,15} But the diagnostic accuracy of u/sound is usually operator dependant and inter observer bias may become a problem. In the current study a total of 151 (60.4%) patients had a positive u/sound test and out of these, 146 (58.4%) were true positive on the basis of histopathology report. The sensitivity and specificity of u/sound in this study was 62.66% and 70.59% respectively while PPV and NPV was calculated as 96.69% and 12.12% respectively.

S Scammell et al, Hemant N. et al, Mohammad A. A. M. et al Tariq W. K. et al observed that Ultrasonography can be used liberally to aid in the decision making process of equivocal and complicated cases of appendicitis and thereby reduce the negative appendectomy.^{2, 3, 6, 12, 15}

S.ali et al also concluded that U/sound promises to be the investigation of choice because this test is non-invasive and can be of particular help in pregnancy but its main disadvantage lies in the fact that it requires special equipment and special expertise.^{5, 22} While in contrast to that the study by SR Markar et al concludes that the use of pre-operative imaging and laparoscopy in patients with acute appendicitis failed to reduce negative appendectomy, perforation and complications rates.³⁰

A positive ultrasound almost always rules in acute appendicitis. At the same time it is important to note that the false negative test in 87 histopathology cases show that u/sound can easily miss a genuine diagnosis if patients are subjected to ultrasound alone. Out of the 233 histopathology positive patients 230 were declared positive on naked eye examination at the operation table. Hence it could be suggested that in settings where facility for histopathology is not available surgeon could

safely label the patient's finding accordingly. But a limitation to this may of course include the surgeon's skills knowledge and experience. Since this study was meant to find out the diagnostic accuracy by combining of all of the above mentioned tests, quite interesting results were encountered. As a matter of fact, combined sensitivity and specificity is simply not an average of all tests neither could they be calculated using cross tabulation, the statistical method of addition or multiplication rule of probability that is used in such cases was employed. A highly sensitive test if negative rules out the disease and highly specific test if positive rules in the disease.³¹

Using this statement as a guide in the current study it was observed that if addition rule of probability was used for both combined sensitivity and specificity the diagnostic accuracy significantly increased and this allows us to invoke the rule out or rule in method for the diagnosis of acute appendicitis. The resultant combined sensitivity and specificity for TLC, CRP, neutrophil% and ultrasound was calculated as 99.17% and 98.45% respectively. The combined sensitivity of TLC, CRP, neutrophil% and u/sound is definitely high from that of TLC (77.68%), neutrophil% (69.96%), CRP (67.10%) and u/sound (62.66%) alone and also from combining two of the tests i.e. TLC and CRP (92.63%) and combining three tests i.e. TLC , CRP, and neutrophil% (77.82%) .

The combined specificity of TLC, CRP, neutrophil% and u/sound is high from that of TLC (64.71%), neutrophil% (58.82%), CRP (64.71%) and u/sound (70.59%) alone and also from combining two of the tests i.e. TLC and CRP (58.2%) and combining three tests i.e. TLC , CRP, and neutrophil% (82.8%) . Shefki Xharra et al in their study also concluded that The combination of CRP, TLC, and the neutrophil percentage has greater diagnostic accuracy in acute appendicitis than individual tests This combination significantly decreases false positive and false negative diagnosis, but none of these is 100% diagnostic for acute appendicitis.¹⁸

While in contrary to that S.Ali et al in their study reported that the sensitivity decreased when TLC, Neutrophil%, CRP and U/Sound were used together to predict appendicitis but the specificity increased markedly (95%) (using the rule in method) indicating the fact that acute appendicitis is unlikely when all these tests are simultaneously negative.²²

Unfortunately, no local study could be found to co-relate the results with. Also in the previous studies, the statistical measure employed for calculating diagnostic accuracy of combined tests are not clear therefore the inferences drawn are based on mere statistical knowledge of multiple testing

taking each test independent of each event. One criticism that might arise in the current study is that for calculating the final sensitivity and specificity for combined tests the addition rule of probability was employed since the multiplication rule of probability yields undesirably low values when used for either of the combined sensitivity or specificity. In case of the current study combined sensitivity and specificity after using the multiplication rule became 22.75% and 17.38% respectively which of course is not what is desired to diagnose a case of acute appendicitis. Though when the addition rule is used for either one i.e. Sensitivity or specificity the vice versa is calculated as the product of either sensitivity or specificity of all tests depending on whether we are interested in the rule in or rule out the method in the diagnosis of a disease.³¹

As far as tests performed for clinical accuracy are concerned it doesn't matter what method we employ; a higher sensitivity or a higher specificity, either way the diagnostic accuracy is significantly improved. And definitely adds to the clinical diagnosis.³¹ Combining tests yielded promising results therefore it is humbly recommended that further studies on combining blood tests with imaging modalities should be considered. Finding similar results will help to improve the diagnostic protocol for acute appendicitis and thereby help in reducing the rate of unnecessary appendectomies. In the current study it was also observed that it was highly unlikely for a patient with acute appendicitis to have all the tests negative, therefore a patient with all the tests negative could be safely observed, treated conservatively or sent home. But once again it should be emphasized that clinical diagnosis should be preferred over equivocal results of the tests and these test should not replace but rather compliment the clinical diagnosis of acute appendicitis.

In summary, this study provides promising results for accuracy of combined tests. A basic point of view about the calculation, the advantages and limitations of these measures have been put forward. Further work/studies in this regard may prove helpful in adding useful information to the national as well as international health care system.

Conclusion

The study concluded that there was a predominance of male patients as compared to female patients as observed in the study. TLC had the highest sensitivity of all the four tests but TLC alone can't be used as a diagnostic test and clinical findings should be preferred over the TLC results. The study concluded that TLC and neutrophils% were found to be a bit more sensitive than CRP in diagnosing acute appendicitis but its role

as a predictor of severity of the disease has to be studied further in order to correlate different grades of appendicitis with different values of CRP rather than labeling it as positive or negative test based on a certain cut off value. Ultrasound was observed to have the best specificity of all the four tests. Combining all of the tests yields improved clinical accuracy. Though acute appendicitis is usually a clinical diagnosis and requires significant skills and knowledge, in order to avoid unnecessary appendectomies adding these tests will compliment them but definitely cannot replace the clinical skills. Further work on combined tests is recommended to order to re-define the diagnostic protocol for acute appendicitis.

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