Emergency. 2018; 6 (1): e51

OPEN ACCESS

ORIGINAL RESEARCH

Serum Procalcitonin and Lactoferrin in Detection of Acute Appendicitis; a Diagnostic Accuracy Study

Mohammad Reza Motie[1], Anvar Soleimani[2,3], Arash Soltani[2,3], Seyed Isaac Hashemy[1,2]*

1. Surgical Oncology Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.

Received: Apirl 2018; Accepted: August 2018; Published online: 25 August 2018

Abstract: **Introduction:** Finding an accurate diagnostic test can reduce the rate of unnecessary abdominal surgery in cases of suspected acute appendicitis (AA). This study aimed to evaluate the diagnostic value of serum lactoferrin (LF) and procalcitonin (PCT) in detection of patients with acute appendicitis. **Methods:** In this diagnostic accuracy study, screening performance characteristics of PCT and LF were calculated in patients suspected with acute appendicitis and healthy volunteers as control group. **Results:** 131 cases participated (61 as case and 70 as control). The mean serum level of LF (0.9±0.14 vs 0.2±0.13 μg/ml; p 0.0001) and PCT (0.15±0.21 vs 0.11±0.02 ng/dl; p = 0.02) were significantly higher in patients suspected with AA. The AUC of PCT and LF were 0.46 (95% CI: 0.31-0.61) and 0.61 (95%CI: 0.47 - 0.76), respectively. At a 0.90 μg/ml cut-off value, LF had 77% (95 % CI: 63 - 91) sensitivity and 43% (95% CI: 31 - 55) specificity. Also, at a 0.11 ng/dl cut-off value, PCT had 41% (95% CI: 26 - 56) sensitivity and 69% (95% CI: 53 - 85) specificity. **Conclusion:** Based on the main finding of present study, the overall accuracy of serum PCT and LF in detection of patients with acute appendicitis are in poor to failed range and it seems that they could not be considered as good screening tools for this purpose.

Keywords: Diagnosis; Marker; Procalcitonin; Lactoferrin; Acute Appendicitis

Cite this article as: Motie M R, Soleimani A, Soltani A, Hashemy S I. Serum Procalcitonin and Lactoferrin in Detection of Acute Appendicitis; a Diagnostic Accuracy Study. Emergency. 2018; 6(1): e51.

1. Introduction

Acute appendicitis (AA) is the most common abdominal emergency worldwide with a significant morbidity and mortality rate. It requires abdominal surgery with an overall lifetime risk of 8.6% in males and 6.7% in females (1, 2). The diagnosis of acute appendicitis is challenging and may be the most common diagnostic problem in clinical surgery (3). Late diagnosis and delay in surgery of AA may result in ruptured appendicitis, systemic septic complications and a longer stay at hospital.

Abdominal pain is a common cause for visiting in the emergency department (ED). The initial clinical sign of appendicitis is pain in the lower right quadrant of abdomen or periumbilical pain, followed by nausea, vomiting and fever. This event occurs approximately in 50% of adults (4). However, the mentioned symptoms are associated with a variety of pathologies and they do not have sufficient specificity and sensitivity; therefore, clinical diagnosis of AA from other pathologies can be challenging and may finally lead to unnecessary appendectomy. The diagnosis of AA is usually confirmed by conventional biomarkers including C-reactive protein (CRP), white blood cell count (WBC), body temperature (BT) and absolute neutrophil count (ANC) (5).

Since advances in radiographic imaging, such as ultrasound and CT scan, have improved the accuracy of diagnosis of AA, the rate of unnecessary appendectomy has decreased.

However, studies have shown that the prevalence of unnecessary appendectomy is around 45%, which is particularly common in females (1, 4). Therefore, researchers have tried for decades to find diagnostic markers to increase the accuracy and safety of clinical diagnosis of AA.

Several studies have shown that serum level of procalcitonin (PCT) and lactoferrin (LF) increase during bacterial infec-

***Corresponding Author:** Seyed Isaac Hashemy; Surgical Oncology Research Centre, Mashhad University of Medical Sciences, Mashhad, Iran. Email: hashemyi@mums.ac.ir Tel: +98-9158112664

tions (2, 8, 9). PCT, a precursor of calcitonin, is secreted by C cells of thyroid gland under normal conditions, and it has been demonstrated to be one of the most important laboratory markers for the presence of fungal and bacterial diseases. Unlike other clinical biomarkers such as CRP, the level of procalcitonin does not change in patients with non-bacterial and viral infections, which makes it an appropriate biomarker for AA (10, 11). A lot of studies have assessed the value of PCT for diagnosing and determining the severity of acute appendicitis, but the results have been contradictory and the diagnostic value of procalcitonin in appendicitis is still unclear (8, 12).

LF is a glycoprotein with a molecular weight of 78 KD. It has antibacterial properties and acts against a wide range of gram-positive and gram-negative bacteria. LF is released exactly like other neutrophil proteins following neutrophil activity, as seen in individuals with inflammatory bowel disease, and it quickly appears in the feces (13, 14). Both PCT and LF are released after neutrophil activation, and their plasma level can be measured in the serum or plasma (15).

Based on the above-mentioned points, we decided to evaluate the diagnostic value of serum LF and PCT in discrimination of patients with acute appendicitis among suspected cases.

2. Methods

2.1. Study design and setting

In this diagnostic accuracy study, patients suspected with acute appendicitis (based on a primary diagnosis by physicians), who had been referred to Imam Reza Hospital, Mashhad, Iran, from September 2015 to September 2017 were included. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences and written informed consent was taken from each patient.

2.2. Participants

Patients more than 16 years old with acute abdominal pain, who had been referred to emergency department of mentioned hospital, were included using convenience sampling method. In addition, 70 healthy volunteers including males and females whose sex and age were matched with patients were enrolled as the control group. Patients with any evidence of other diseases such as cancer, inflammatory bowel disease, consumption of steroidal or non-steroidal anti-inflammatory drugs and antibiotics before their hospitalization, patients without any pathological findings, patients with reactive follicular hyperplasia or chronic appendicitis, and those with certain infectious diseases were excluded.

2.3. Procedure

Suspected patients were primarily detected at the emergency triage unit, and then were examined by a surgeon. Alvarado score (16) was recorded for all patients, and appendectomy was carried out based on the routine surgery department protocol. Blood samples for laboratory tests were collected from all patients on admission before appendectomy. Serum PCT and LF levels were measured by commercially available ELISA kits from HyCult Biotechnology (Cat # HK329-01) for LF and abcam (Cat #ab221828) for PCT. After appendectomy, a histopathology study was conducted on all samples, and the results were categorized into one of the 5 following groups by a pathologist: patients without any pathological findings (Group 1); patients with reactive follicular hyperplasia or chronic appendicitis (Group 2); patients with acute appendicitis, intact appendix mucosa and a mild to moderate infiltration of the inflammatory cells (Group 3); patients with macroscopically or histologically perforated acute appendicitis, or perforated appendix mucosa accompanied by a strong pan-mural infiltration of the inflammatory cells (Group 4); and finally, patients with acute necrotizing appendicitis (Group 5). The Alvarado scores less than 4 were defined as unlikely in terms of appendicitis, scores of 5-6 as poor possibility of appendicitis, scores of 7-8 likely and scores of 9-10 were described as high possibility of appendicitis.

2.4. Reference test

The surgical histopathology finding was used as the confirmative test for diagnosis of acute appendicitis.

2.5. Data gathering

Demographic information (age, sex), vital signs (temperature, heart rate, respiratory rate), white blood cell count (WBC), neutrophil count, serum level of LF and PCT, patients' Alvarado score, as well as the results of surgical histopathology of appendix were recorded for all participants using a pre-designed checklist. There were no missing data. All data were collected prospectively by a trained pathology resident.

2.6. Statistical Analysis

Due to the small sample size of our study, bootstrap resampling method was carried out to obtain 95% bias-corrected confidence intervals for each area under the ROC curve (AUC) (17). An evaluation for the cut-off value of the biomarkers for the acute appendicitis diagnosis was defined by the Youden index (18). Statistical tests (chi-squared, Fisher's exact, logistic regression and independent t-test as well as Mann Whitey U test (non-parametric independent-paired comparison)) were used for analyses. Receiver op-

erator characteristic (ROC) curves were drawn to calculate the diagnostic accuracy of the studied serum biomarkers (LF, PCT) with 95% confidence interval (CI). A p value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS11 software, and data were presented as mean ± standard deviation or frequency (%). The correlation of LF, PCT with pathology and Alvarado scores as well as WBC and neutrophil counts was investigated in suspected acute appendicitis cases.

3. Results

3.1. Baseline characteristics

105 cases were detected as patients suspected to have acute appendicitis, 80 of which were eligible for inclusion in the study. 19 (18.1%) patients were pathologically categorized into non-acute group and 61 patients were confirmed as acute appendicitis based on surgical histopathology. Baseline characteristics of studied patients are listed in Table 1. The group suspected with AA and control group were similar regarding mean age (26.32±10.67 vs 28.97±9.63; p = 0.115) and male to female ratio (34/27 vs 34:36; p = 0.319). The mean serum level of LF (0.9±0.14 vs 0.2±0.13 μg/ml; p 0.0001) and PCT (0.15±0.21 vs 0.11±0.02 ng/dl; p = 0.02) were significantly higher in patients suspected with AA.

3.2. Relationships

There was no correlation between serum PCT level and Alvarado score (r= 0.12, p=0.5), pathology grade (r=0.01, p=0.31), WBC count (r= 0.06, p=0.57), and neutrophil count (r= 0.13, p=0.27). In addition, there was no correlation between serum LF level and Alvarado score (r= 0.21, p=0.12), pathology grade (r= 0.19, p=0.10), WBC count (r= 0.13, p=0.23), and neutrophil count (r= 0.17, p=0.15). The correlation coefficient between procalcitonin and lactoferrin was 0.02 (P=0.834).

3.3. Screening performance characteristics of evaluated parameters

The AUC of PCT and LF were 0.46 (95% CI: 0.31-0.61) and 0.61 (95%CI: 0.47 - 0.76), respectively.

At a 0.90 μg/ml cut-off value, LF had 77% (95% CI: 63 - 91) sensitivity, 43% (95% CI: 31 - 55) specificity, 0.71 (95% CI: 0.65-0.78) Positive predictive value, 0.29 (CI 95%:0.23-0.35) negative predictive value, 1.35 (95% CI: 1.12-1.58) positive likelihood ratio, and 0.53 (95% CI: 0.46-0.60) negative likelihood ratio.

Also, at a 0.11 ng/dl cut-off value, PCT had 41% (95% CI: 26 - 56) sensitivity, 69% (95% CI: 53 - 85) specificity, 0.45 (95% CI: 0.38-0.52) positive predictive value, 0.04 (CI 95%: 0.02-0.06) negative predictive value, 1.32 (95% CI: 1.10-1.60) positive likelihood ratio, and 0.85 (95% CI: 0.55-1.25) negative likelihood ratio. Table 2 and figure 1 show the screening performance characteristics of WBC, neutrophil count, and Alvarado score for comparison with PCT and LF.

Table 1: Baseline Characteristics of patients suspected with acute appendicitis (AA) (n=80)

Baseline Characteristics	Suspected to AA
Age (year)	
Mean ± SD	26.32±10.67
Sex	
Male/Female ratio	34:27
Temperature (C)	
Mean ± SD	37.22± 0.63
WBC (10^3 mm^3)	
Mean ± SD	13.28±4.1
Neutrophil (%)	
Mean ± SD	76.05±16
Symptoms n (%)	
Shifting pain	62 (77.5)
Nausea-vomit	67 (83.8)
Anorexia	69 (86.3)
Lactoferrin (μg/ml)	
Mean ± SD	0.9±0.14
Procalcitonin (ng/dl)	
Mean ± SD	0.15±0.21
Alvarado-score n (%)	
<4	1 (1.3)
5-6	20 (25)
7-8	26 (32.5)
9-10	33 (41.3)
Pathology score n (%)	
1	-
2	19 (23.8)
3	10 (12.5)
4	48 (60)
5	3 (3.8)

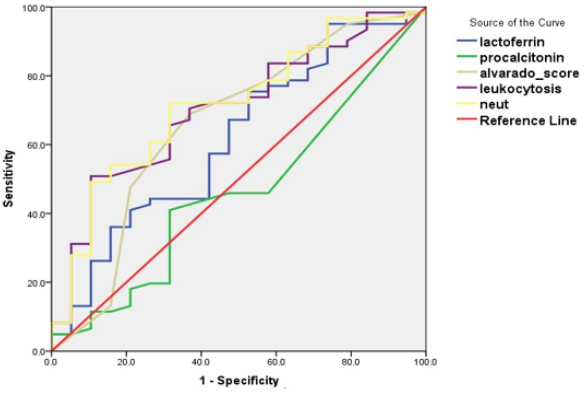

Figure 1: Area under the ROC curve of lactoferrin, procalcitonin, white blood cell count, neutrophil count, and Alvarado score in detection of patients with acute appendicitis.

Table 2: Screening performance characteristics of lactoferrin (μg/ml), procalcitonin (ng/dl), white blood cell (WBC) count ($\times 10^3 mm^3$), neutrophil count, and Alvarado score in detection of patients with acute appendicitis

Variable	Cut-off	Sensitivity	Specificity	AUC
Lactoferrin	0.90	77(63- 91)	43(31-55)	0.61 (0.47 - 0.76)
Procalcitonin	0.11	41(26-56)	69(53-85)	0.46 (0.31-0.61)
WBC count	10	85(72-98)	45(32-58)	0.70 (0.57-0.83)
Neutrophil	75	72(60-84)	69(52-86)	0.71 (0.58-0.84)
Alvarado	7	95(91-99)	22(16-28)	0.66 (0.50-0.81)

All measures were presented with 95% confidence interval. AUC: area under the ROC curve.

4. Discussion

Diagnosis of patients with appendicitis is a big challenge in Emergency Department; therefore, a lot of negative appendectomies have been observed. A correct and early diagnosis of the disease is vital to decrease its morbidity of perforated cases and also to prevent unnecessary surgery for misdiagnosed cases (6). In this regard, a lot of clinical studies have been carried out in order to identify gold markers to reach a better diagnosis. The most accurate clinical biomarkers presented for diagnosis of this disease are CRP and WBC. However, they do not have the ability to discriminate all true patients among suspected subjects (8, 19). In our study, we measured the serum level of lactoferrin and procalcitonin between suspected acute appendicitis cases and healthy controls. The comparison of these biomarkers showed a significant difference between patients with acute appendicitis and healthy controls.

In consistence with our study, another study on new biomarkers for acute appendicitis showed a significant difference of lactoferrin between acute appendicitis and healthy controls (9). In another study, the plasma level of procalcitonin in patients with confirmed acute appendicitis was reported to be more than the control group (0.5 ng/ml serum level for patients) (20). We evaluated the correlation of lactoferrin and procalcitonin with eachother and other criteria including WBC, neutrophil count and Alvarado score. We could not find any correlation between these parameters.

We separated the acute appendicitis cases from non-appendicitis via pathology score after appendectomy. ROC curve analysis showed the best accuracy test for diagnosis of appendicitis is neutrophil count with the AUC of 0.71 and at a defined cut-off of 75%; sensitivity and specificity were 72% and 69%, respectively. The accuracy of test for lactoferrin and procalcitonin were 0.61 and 0.46, respectively.

Since AUC represents the accuracy of the test and AUC of less than 0.5 is not statistically appropriate for diagnosis of disease, the serum level of procalcitonin is not a good diagnostic factor.

However, in one study procalcitonin is introduced as a diagnostic marker for acute appendicitis with a sensitivity of 95.65% and a specificity of about 100% (11). Moreover,in another study the PCT value of > 0.5 ng/ml is shown to be useful for diagnosis of appendicitis with 73% sensitivity and 94% specificity (21).

Khan et al. showed that the serum level of procalcitonin in children with suspected acute appendicitis is higher than control group (1.12 ng/ml ± 3.28 versus 0.45 ng/ml ± 1.12), but it is not statistically significant (p=0.3) (22). The interesting point about procalcitonin is the high specificity in all studies that suggest it as a diagnostic biomarker along with other parameters with high sensitivity such as WBC, CRP and neutrophil count.

The accuracy test for lactoferrin was acceptable (AUC > 0.5) with 77% sensitivity and 43% specificity in determined cut-off of 0.9μg/ml. Lactoferrin is more accurate and sensitive than procalcitonin for diagnosis of acute appendicitis. Sevgi et al. reported a good accuracy (AUC=0.79) for lactoferrin between cases with uncomplicated appendicitis and non-appendicitis (13). Our results showed that lactoferrin is less accurate than WBC, neutrophil and Alvarado score. In our study, both WBC and Alvarado score had better accuracy, specificity and sensitivity as well. Alvarado score with the cut-off of 7 points has the highest sensitivity (95%) while the specificity was just 22%. It has been shown that the sensitivity and specificity of the Alvarado score vary, ranging from 60% to 80% in different studies (23-26). Apparently, Alvarado score has more benefits than biomarkers, because whenever the patient's condition alters, this scoring system can be evaluated more rapidly, repeatedly and easily. Nevertheless, the combination of both Alvarado score and biomarkers such as PCT and LF will be more accurate in diagnosis of patients with appendicitis in the emergency department.

We could find a significant difference between serum level of lactoferrin and procalcitonin between our patients and healthy controls; however, the measured parameters did not have any correlation with each other. Neutrophil and WBC count seem to be better diagnostic markers for acute appendicitis compared to lactoferrin and procalcitonin. Procalcitonin and lactoferrin might have the potential to be used in a panel with WBC and CRP in order to decrease the number of false positive and false negative cases.

5. Limitation

It should be noted that the result of the present study has been obtained in light of some limitations. First, we had a small sample size and it did not allow us to make any generalization. Second, the measurement of those parameters is expensive and takes a few hours, which may be an important time period in the emergency department. Third, the cut-off value presented in our study may not work as well in another study with a different case mix. Lastly, Procalcitonin may not increase significantly until 8–12 h. Therefore, for patients admitted early to the emergency department, serum PCT may not indicate the clinical severity.

6. Conclusion

Based on the main finding of present study, the overall accuracy of serum PCT and LF in detection of patients with acute appendicitis are in poor to failed range and it seems that they could not be considered as good screening tools for this propose.

7. Appendix

7.1. Acknowledgements

We are thankful to all volunteers who participated in this study. We also acknowledge the financial support of the Research Council of Mashhad University of Medical Sciences for this project.

7.2. Authors' contribution

All the authors participated in the research design and contributed to different sections of the research. The project was performed by Anvar Soleimani. Statistical analyses were performed by Arash Soltani. Management of the project and revision of the manuscript were performed by Mohammad-Reza Motie and Seyed Isaac Hashemy.

7.3. Funding/Support

This project was supported by the Research Council of Mashhad University of Medical Sciences (Grant number: 900647).

7.4. Conflict of interest

There is no competing interest to declare in this manuscript.

References

1. Addiss DG, Shaffer N, Fowler BS, Tauxe RV. The epidemiology of appendicitis and appendectomy in the United States. Am J Epidemiol. 1990;132(5):910-25.
2. Schellekens DH, Hulsewe KW, van Acker BA, van Bijnen AA, de Jaegere TM, Sastrowijoto SH, 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.
3. Smink DS, Finkelstein JA, Garcia Pena BM, Shannon MW, Taylor GA, Fishman SJ. Diagnosis of acute appendicitis in children using a clinical practice guideline. J Pediatr Surg. 2004;39(3):458-63.
4. Flum DR, Morris A, Koepsell T, Dellinger E. Has misdiagnosis of appendicitis decreased over time? A population-based analysis. JAMA. 2001;286(14):1748-53.
5. Humes DJ, Simpson J. Clinical Presentation of Acute Appendicitis: Clinical Signs–Laboratory Findings–Clinical Scores, Alvarado Score and Derivate Scores. In: Keyzer C, Gevenois PA, editors. Imaging of Acute Appendicitis in Adults and Children. Berlin, Heidelberg: Springer Berlin Heidelberg; 2012. p. 13-21.
6. Mostbeck G, Adam EJ, Nielsen MB, Claudon M, Clevert D, Nicolau C, et al. How to diagnose acute appendicitis: ultrasound first. Insights Imaging. 2016;7(2):255-63.
7. Petroianu A. Diagnosis of acute appendicitis. Int J Surg. 2012;10(3):115-9.
8. Yu CW, Juan LI, Wu MH, Shen CJ, Wu JY, Lee CC. Systematic review and meta-analysis of the diagnostic accuracy of procalcitonin, C-reactive protein and white blood cell count for suspected acute appendicitis. Br J Surg. 2013;100(3):322-9.
9. Thuijls G, Derikx JP, Prakken FJ, Huisman B, van Bijnen Ing AA, van Heurn EL, et al. A pilot study on potential new plasma markers for diagnosis of acute appendicitis. Am J Emerg Med. 2011;29(3):256-60.
10. Al-Nawas B, Krammer I, Shah PM. Procalcitonin in diagnosis of severe infections. Eur J Med Res. 1996;1(7):331-3.
11. Chandel V, Batt SH, Bhat MY, Kawoosa NU, Yousuf A, Zargar BR. Procalcitonin as the biomarker of inflammation in diagnosis of appendicitis in pediatric patients and prevention of unnecessary appendectomies. Indian J Surg. 2011;73(2):136-41.
12. Yamashita H, Yuasa N, Takeuchi E, Goto Y, Miyake H, Miyata K, et al. Diagnostic value of procalcitonin for acute complicated appendicitis. Nagoya J Med Sci. 2016;78(1):79-88.
13. Sarsu SB, Erbagci AB, Ulusal H, Karakus SC, Bulbul OG. The Place of Calprotectin, Lactoferrin, and High-Mobility Group Box 1 Protein on Diagnosis of Acute Appendicitis with Children. Indian J Surg. 2017;79(2):131-6.
14. Green I, Kirkpatrick CH, Dale DC. Lactoferrin–specific localization in the nuclei of human polymorphonuclear neutrophilic leukocytes. Proc Soc Exp Biol Med. 1971;137(4):1311-7.
15. Langhorst J, Elsenbruch S, Koelzer J, Rueffer A, Michalsen A, Dobos GJ. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-

elastase, CRP, and clinical indices. Am J Gastroenterol. 2008;103(1):162-9.
16. Alvarado A. A practical score for the early diagnosis of acute appendicitis. Ann Emerg Med. 1986;15(5):557-64.
17. Pepe MS, Longton G. Standardizing diagnostic markers to evaluate and compare their performance. Epidemiology. 2005;16(5):598-603.
18. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. Biom J. 2005;47(4):458-72.
19. Mikaelsson C, Arnbjornsson E. The value of C-reactive protein (CRP) determinations in patients with suspected acute appendicitis. Ann Chir Gynaecol. 1984;73(5):281-4.
20. Wu JY, Chen HC, Lee SH, Chan RC, Lee CC, Chang SS. Diagnostic role of procalcitonin in patients with suspected appendicitis. World J Surg. 2012;36(8):1744-9.
21. Kafetzis DA, Velissariou IM, Nikolaides P, Sklavos M, Maktabi M, Spyridis G, et al. Procalcitonin as a predictor of severe appendicitis in children. Eur J Clin Microbiol Infect Dis. 2005;24(7):484-7.
22. Khan AN, Sawan A, Likourezos A, Schnellinger M, Garcia E. The usefulness of procalcitonin in the diagnosis of appendicitis in children: a pilot study. Emerg Med Int. 2012;2012:317504.
23. Bundy DG, Byerley JS, Liles E, Perrin EM, Katznelson J, Rice HE. Does this child have appendicitis? JAMA. 2007;298(4):438-51.
24. B. ARE. Meta-analysis of the clinical and laboratory diagnosis of appendicitis. BJS. 2004;91(1):28-37.
25. Abdeldaim Y, Mahmood S, Mc Avinchey D. The Alvarado score as a tool for diagnosis of acute appendicitis. Irish medical journal. 2007;100(1):342.
26. Wu J-Y, Chen H-C, Lee S-H, Chan R-C, Lee C-C, Chang S-S. Diagnostic Role of Procalcitonin in Patients with Suspected Appendicitis. World J Surg. 2012;36(8):1744-9.