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ORIGINAL PAPER

LEUKOCYTES, C-REACTIVE PROTEIN AND INTERLEUKIN-6 IN ACUTE APPENDICITIS IN CHILDREN: DIAGNOSTIC VALUE AND ASSOCIATION WITH HISTOLOGICAL FINDINGS

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ABSTRACT

Background: Many efforts have been made to find diagnostic tools that would help select children with clinical signs of acute appendicitis who would need immediate appendectomy and to find tools that would reduce the numbers of negative appendectomies.
Aim: We aimed to evaluate diagnostic values of leukocyte count, level of C-reactive protein and interleukin-6 for negative appendicitis in children with high clinical probability for appendicitis (Alvarado score>7) using degree of histological findings of appendix as a gold standard.
Methods: We analyzed 80 patients of both genders, younger than 15 years, with Alvarado score>7, who underwent open appendectomy with subsequent histological analysis of the removed appendices. We sampled 20 consecutive cases without pathohistological signs of inflammation (group I), 20 cases with incipient inflammation (group II), 20 cases with phlegmonous inflammation (group III) and 20 cases with signs of perforated appendix (group IV). Prior to appendectomy, a peripheral blood was sampled and sent for analysis of leukocyte count and C-reactive protein and interleukine-6 level. We compared values of all 3 measured parameters according to histological findings; we also used Receiver Operating Characteristics (ROC) analysis in order to evaluate diagnostic thresholds for detecting the histological signs of appendicitis.
Results: The lowest values of all analyzed parameters were found in the group of negative appendicitis while highest were observed in the group of perforated appendicitis. We have observed significant group differences in all three analyzed parameters which corresponded to the degree of histological inflammation ($p<0.001$). ROC analysis demonstrated that interleukine-6 had the best diagnostic performance in detecting patients with histological signs of appendicitis (AUROC=0.99; 95% CI=0.99-1.00) when compared to CRP and leukocyte count ($p<0.05$). There was no significant difference in diagnostic performance between CRP and leukocytes counts ($p=0.35$).
Conclusion: Leukocyte count, CRP and interleukine-6 are very useful markers which may help in diagnostics and differentiation of phlegmonous and perforated appendicitis. In patients with high probability of appendicitis, the measurement of interleukine-6 may help in better patient selection.

Keywords: *acute appendicitis; children; interleukine-6; C-reactive protein; leukocyte count*

INTRODUCTION

Diagnosis of acute appendicitis in children is more difficult than in adults. Children may not be able to give accurate information about their sickness, beginning and characteristics of pain and the other symptoms. Widely used therapy for suspected appendicitis in children is appendectomy; conservative approach was considered in several studies, yet it is not clearly accepted in pediatric surgery.[1] A delayed diagnosis of acute appendicitis in children does increase the risk for perforation and further complications. However, in children, geriatric patients and female adolescents, the ratio of negative appendectomies can be as high as 50%.[2]

Table 1. *Comparison of leukocyte, C-reactive protein and interleukine-6 values between 4 groups of patients with different histological findings after appendectomy*

Parameter	Histological findings of removed appendices				Kruskal-Wallis test
	Negative	Incipient appendicitis	Phlegmonous appendicitis	Perforated appendicitis	
***Leukocyte (10^9/l)**	7.6; 4.7 - 14.1	11.6; 6.1 - 20.2	13.4; 5.4 - 22.0	16.0; 8.9 - 27.7	X^2=25.90; df=2; p<0.001
*** C-reactive protein (0,3 - 3mg/ml)**	3.45; 6.0 - 59	15.7; 1.1 - 48.0	7.8; 2.4 - 92.4	61.9; 4.8 - 200	X^2=30.23; df=2; p<0.001
*** Interleukine-6 (0,7 - 3,7pg/ml)**	0.7; 0.7 - 5.5	11.6; 5.1 - 27.1	15.2; 6.3 - 30	55.2; 9.4 - 172	X^2=61.02; df=2; p<0.001

One of clinical scores that can help in diagnosis of acute appendicitis is Alvarado score, based on the estimate of several clinical signs.[3] Alvarado score can take values from 1 to 10 points, so patients with a total count of nine or ten usually do have acute appendicitis. Patients with a score of seven or eight have high probability of having acute appendicitis.

In the last couple of years, a great attention has been given to the mediators of serum inflammation like white blood cells count (Lkc), C-reactive protein (CRP) and interleukin-6 (IL-6) in the diagnostics of acute appendicitis and preventions of negative laparotomies.[4]

Many efforts have been made to find a way to reduce the numbers of negative appendectomies. On the other hand, equally valuable are indicators of the necessity for the immediate appendectomy. Although abdominal ultrasound, CT scan or explorative laparoscopy are helpful, non invasive method such as use of markers of inflammation could give us information with the same level of accuracy. This could be useful in clinical decision making i.e. when to make appendectomy, especially in patients with phlegmonous or perforated appendix.

We aimed to evaluate diagnostic values of leukocyte count, level of C-reactive protein and interleukin-6 for negative appendicitis in children with high clinical probability for appendicitis (Alvarado score>7) using degree of histological findings on appendix as a gold standard.

PATIENTS AND METHODS

Patients

We prospectively selected 80 consecutive patients of both gender, younger than 15 years, which were treated at the Department of Pediatric Surgery, University Clinical Center Tuzla from January 2007 to January 2008. Selection process was based on histological criteria and is described below. All patients underwent the same preoperative assessment which included a thorough history, clinical exam, basic lab tests, anesthesiology assessment, preoperative antibiotics and depending of operative finding, a postoperative course of antibiotics.

Methods

Indications for surgery were based on history, clinical findings, physical exam and laboratory workup. To estimate the need for surgery, we used the Alvarado score. Symptoms, signs and lab findings were scored by Alvarado score by using usual methodology described by original authors.[3] If Alvarado score was higher than 7, we proceeded with appendectomy. Alvarado score<7 was our main exclusion criteria. Unfortunately, routine 24-hours access to CT scan and/or ultrasound was not available at the time in our hospital.

Prior to appendectomy, a peripheral blood was sampled and sent for analysis of leukocyte count and CRP level. Additionally, 5 cc of serum was frozen and kept on -20°C for later determination of interleukin-6 level, which we analyzed after collecting samples from all patients. Leucocyte count was measured with hematol-

ogy counter Sysmex KX-21 (Japan), CRP was measured with Dimension system RxL Max (DADE Behring) and interleukin-6 was measured with IL6 Quaulikine IL6 (R&D Systems Minneapolis, USA).

All patients were operated with open appendectomy in general anesthesia with tracheal intubation using standard anesthesiology protocol. Removed appendix was immediately sent for histology analysis. After standard staining, microscopic examination of tissue samples was done by a single experienced pathologist. We recruited 20 consecutive cases without signs of inflammation (group I), 20 cases with pathological signs of incipient inflammation (group II), 20 cases with signs of phlegmonous inflammation (group III) and 20 cases with signs of perforated appendix (group IV).

Negative appendectomy cases were considered as those where there were no signs of inflammation in the wall of appendix. Incipient inflammation was considered in cases with shallow erosions and/or with presence of focal and rare polymorphonuclear infiltrates in the wall of appendix. Phlegmonous inflammation was considered where findings of dense infiltrates of polymorphonuclear leukocytes in all part of appendix wall were found with or without findings of pus or necrosis. Perforated appendix was assumed in cases of putrid and necrotizing inflammation all layers of appendix wall with microscopic signs of wall penetration.

Statistical analysis

All data were analyzed by using SPSS 15.0 software (SPSS Inc, Chicago, IL, USA). Basic measures of central tendency and dispersion were analyzed for all variables which were also tested for normal distribution by using Kolomogorov-Smirnov Test. Chi-square test was used for comparison of qualitative variables

In addition, Kruskall-Wallis test was used for multiple comparisons between groups. Receiver Operating Characteristic (ROC) analysis was used for testing of diagnostic performance of interleukin-6, CRP and leukocytes in diagnosis of acute appendicitis. Threshold values were tested by using a simple Bayesian analysis. ROC curves were compared using Hanley and McNeil method.[5] All tests were done with the significance level of 95% ($p<0.05$).

RESULTS

Out of 80 patients, 36 (45%) were females. The average age (±SD) in sample was 9.7±2.6 years. Baseline preoperative values of Lkc, CRP and IL-6 in peripheral blood of children with acute appendicitis in relation to microscopic findings of removed appendices are presented in table 1.

The lowest values of all observed parameters were found in group of negative appendicitis while highest were observed in the group of perforated appendicitis. As presented in table 1, we have observed significant group differences in all analyzed parameters.

Analysis of diagnostic performance of leukocyte count in detection of appendicitis has shown that the area under the ROC curve (AUROC) was 0.85 (95% CI=0.765-0.942) – figure 1. The best diagnostic performance has been noted for the threshold value of leukocyte count of 10.5 with the sensitivity of 76.3%, specificity of 80%, positive predictive value of 91.8% and negative predictive value of 53.3%.

ROC analysis was also performed for diagnostic performance of CRP in detection of acute appendicitis – figure 1. The area under the ROC curve was 0.798 (95%

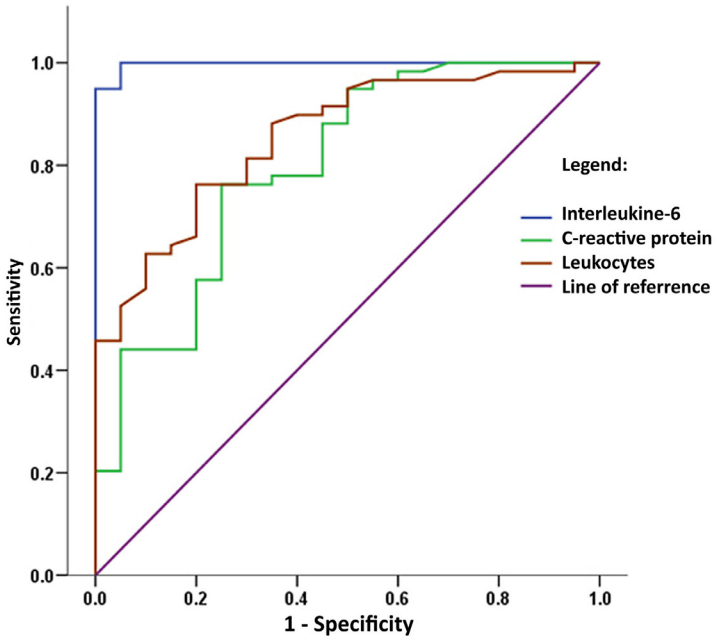

Figure 1. *Comparison of Areas Under the Receiver Operating Charactheristics Curve (AUROC) for leukocyte count, C-reactive protein and interleukine-6 levels in detecting appendicitis.*

CI=0.68-0.92). The best diagnostic performance was found with the CRP threshold value of 7.4 with sensitivity of 76.3%, specificity of 75%, positive predictive value of 90%, and negative predictive value of 51.7%.

The ROC analysis was performed regarding diagnostic performance of IL-6 in detection of acute appendicitis – figure 1. The area under the ROC curve was found to be 0,997 with 95% CI of 0.991 to 1.000, which means that IL-6 had an excellent diagnostic accuracy. The best diagnostic characteristics were found with threshold value of 4.1 with sensitivity of 100%, specificity of 95%, positive predictive value of 98.3%, and negative predictive value of 100%.

We compared AUROC values of all 3 evaluated parameters (figure 1). Diagnostic performance of IL-6 was significantly better $p<0.001$ with average difference between AUROC (±SE) of 0.20±0.05 and 0.14±0.04, respectively in comparison with CRP and Lkc. There was no significant deference in diagnostic performance between Lkc and CRP ($p=0.35$; with average difference between AUROC=0.06±0.06).

DISCUSSION

Acute appendicitis is the most common reason for urgent operative treatment in children and one of most common causes of abdominal pain. Abdominal pain is a very frequent reason that parents decide to bring their children to doctors' office. It may be difficult to differentiate between acute appendicitis and other causes of abdominal pain is some time very hard, especially in small children. This is mainly the reason for high rates (28-57%) of the initial misdiagnosis of acute appendicitis in children.[6] There is no specific diagnostic method for detecting acute appendicitis, but there are several complementary methods which may help in the process of establishing the diagnosis.

More than 250 000 appendectomies are done every year in USA. Of that number, 15% is negative, and around 100 children die due to consequences of wrong diagnosis.[7] Every year, 70 000 patients are discharged from pediatric hospitals after being treated for acute appendicitis or related sickness, costs of which climb to 680 million dollars.[8] In one third of children with acute appendicitis, perforation of appendix occurs prior to operative treatment, mainly in children < 4 years.

The usual laboratory parameters used in diagnostics of acute appendicitis are complete blood count and urine analysis, leukocyte count and C-reactive protein. Erkasap at al.[9] reported that CRP, Lkc and IL-6 can be of particular importance in diagnostics of acute appendicitis.

In our study, we analyzed values of Lkc, CRP and IL-6 levels in patients with negative, incipient, phlegmonous and perforated appendicitis. The values differed to a degree that was directly related to the degree of histological inflammation. The lowest values were observed among those with histologicaly negative appendicitis and the highest among those with perforated appendicitis. Our findings were similar to those observed in other published papers.[9-10]

Andersson et al.[2] reported that elevated Lkc counts are always present in patients with phlegmonous and perforated appendicitis. However, leukocytosis is not a specific parameter for acute appendicitis and it needs to be supported by other diagnostics methods in order to establish the diagnosis of acute appendicitis.

According to Chung et al.[11] diagnostic values of CRP in children have a great significance, especially in cases of perforated appendicitis. According to Chung, values of CRP higher than proposed threshold of 25 mg/L are sign of higher probability of acute appendicitis. Similar results were published by Rodriguez-Sanjuana et al.[12] however their proposed CRP threshold was 17 mg/L. CRP levels lower than this threshold signal that the diagnosis of acute appendicitis is less likely.

On the other hand, other investigators suggest that white blood cell count or CRP values alone do not appear to provide any useful additional information to the surgeon. The sensitivity of the 2 combined tests is extremely high, and normal values of both WBC and CRP are very unlikely in pathologically confirmed appendicitis.[13]

Gurleyik et al.[14] reported that IL-6 level was false negative in many patients with confirmed acute appendicitis. But, it has been proven that high preoperative IL-6 level is associated with perforated appendicitis and higher number of postoperative complications. Paajanena et al.[15] found in their research that in 90% of cases, if Lkc, CRP and IL-6 are negative, there is no acute appendicitis.

Levels of IL-6 were also compared against imaging methods such as ultrasound (US). In two complementary studies by Groselj-Grenc et al. it has been shown that IL-6 had inferior diagnostic accuracy when compared with ultrasonography, but was superior to lipopolysaccharide-binding protein.[16,17] Authors concluded that IL-6 does not significantly improve the diagnosis of AA while ultrasonography was a more accurate diagnostic method than IL-6 serum concentration. Although computed tomography and ultrasonography are widely used as extremely valuable diagnostic procedures in the diagnosis of appendicitis they are, however, used where available. In the settings of the devel-

oping country with limited resources these procedures are not always available - sometimes due to the lack of the hardware and sometimes due to the lack of educated operators. This is where combination of clinical score (Alvarado) and laboratory parameter may be of value.

ROC analysis of diagnostic performance in our study shows that serum IL-6 level has great diagnostic accuracy in diagnosis of the acute appendicitis in children. Similar results were reported by Papanicolau et al.[18] suggesting that IL-6 could be used as a standard method in acute appendicitis diagnostics. We believe that routine use of IL-6 would decrease unnecessary appendectomies, postoperative complications and cut down the costs even IL-6 is expensive marker.

Although our ROC analysis of tested parameters was not entirely appropriate in the context of the experimental design of this particular study, still it yielded several interesting findings. Comparative analysis of ROC curves for diagnostic performance Lkc, CRP and IL-6 demonstrated that diagnostic performance of IL-6 was much better than CRP and Lkc. There was no difference in diagnostic performance between Lkc and CRP. There is an obvious superiority of interleukine-6 in diagnosis of acute appendicitis when compared with other investigated parameters. One must bear in mind that all patients in our study had Alvarado score>7 – which is a criterion for high suspicion of acute appendicitis. Interleukine-6 had AUROC of 0.99, which is an excellent diagnostic performance, implying that it may be of tremendous help in this particular subset of patients and perhaps it should be recommended as a standard part of evaluation in diagnostics of acute appendicitis in children.

There are several limitations of the study that need to be discussed. The sample size is low; however, since we were forced to create numerically equal groups it was not easy to have the sample that will contain sufficient number of negative appendectomies. This was our main limitation factor. We also intentionally selected patients with Alvarado score>7 since this group of patients has the high risk for appendicitis, we agree that it would be interesting to test our parameters in a wider cohort of patients. Yet, we intended to improve the selection of patients for surgery in the clinical settings of high suspicion for appendicitis.

One may also pose a question regarding the possible sample selection bias. In our study the sample included only patients that were operated while we completely omitted those that were not. This may have possible impact on the positive and negative prediction values or in other words PPV may be high. This is true in some way but one must bear in mind that we designed our study in way that would allow us to evaluate value of CRP, IL-6 and leukocyte levels in patients with high clinical suspicion for acute appendicitis; or in other words, patients that would end up being operated. Therefore, our intention was to evaluate the value of all of afore mentioned parameters in reduction of unnecessary surgeries. In this particular setting NPV is of greater interest and we believe that possible sample bias has lower impact on its accuracy.

In this study we focused on diagnostic values of different acute phase inflammatory markers with a particular aim to attempt to facilitate the correct and timely diagnosis of acute appendicitis in children. As we showed, CRP, IL-6 and Lkc are very useful markers which may help in diagnostics and differentiation of phlegmonous and perforated appendicitis. In patients with high probability of appendicitis, measurement of interleukine-6 may help in better patient selection. Choosing patients that need immediate surgery and avoiding to operate those that may have the negative appendectomy is of particular importance in the process of clinical decision making in acute appendicitis in children.

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