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Diagnostic Values of a Single Serum Biomarker at Different Time Points Compared with Alvarado Score and Imaging Examinations in Pediatric Appendicitis

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Background. **A large number of children with right lower quadrant pain are evaluated on a daily basis by primary care clinicians in order to rule out acute appendicitis. The aim of this study was to determine the cutoff values of serum biomarkers, including white blood cell (WBC) count and C-reactive protein (CRP) in predicting pediatric appendicitis based on how long the patients' symptoms were present. We further compared the diagnostic values of these serum biomarkers with the Alvarado score and imaging examinations.**

Materials and Methods. **This prospective study comprised of 594 pediatric patients with suspected appendicitis was conducted at a medical center in Taiwan from 2004 to 2006. Receiver operating characteristic (ROC) curves were used to establish the best cutoff values of serum biomarkers for discriminating pediatric appendicitis. We further analyzed the diagnostic values of the Alvarado score, abdominopelvic computerized tomography (CT), and ultrasonography in predicting appendicitis, and then compared them with our selected serum biomarkers.**

Results. **ROC analysis showed that the best cutoff value of WBC count was 11,000/mm on the first day after onset of symptoms (d 1), and the best cutoff values of CRP concentration were 25 mg/L (d 2) and 89 mg/L (d 3) in diagnosing acute appendicitis. The cutoff values of CRP concentration to indicate perforated appendicitis were 24 mg/L (d 1), 50 mg/L (d 2), and 119 mg/L (d 3). A single serum biomarker at different time points has a diagnostic value, which is as favorable as that of Alvarado score and CT imaging and better than that of ultrasonography in predicting pediatric acute or perforated appendicitis.**

Conclusion. **A single serum biomarker at different time points has a favorable diagnostic value, which is inexpensive, objective, and readily available without the risk of radiation or the need of sedation and is useful for primary caregiver.**

Key Words: **appendicitis; diagnosis; white blood cell; C-reactive protein; Alvarado Score; imaging.**

INTRODUCTION

A large number of children with right lower quadrant pain are evaluated on a daily basis by primary care clinicians in order to rule out acute appendicitis [1]. Despite intensive research and discussion, a rapid accurate diagnosis of pediatric appendicitis remains elusive [2, 3]. The rate of misdiagnosis of pediatric appendicitis ranges from 28% to 57% in children aged 12 y and younger, and the frequency of perforation is nearly 100% in children aged 2 years and younger [4]. In addition, a clinical decision of surgery leads to the removal of a normal appendix in 10% to 20% of cases [5, 6]. Furthermore, a misdiagnosis or delayed diagnosis of acute appendicitis may lead to perforation and peritonitis. Thus, improving diagnostic accuracy is

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desirable both for timely diagnosis and for reducing the number of unnecessary appendectomies.

Comparatively new imaging techniques can be valuable aids in the diagnosis of appendicitis, however, their limited availability in primary healthcare settings and high cost remain problematic [1, 3]. Furthermore, it has been argued that findings of imaging studies should not supersede clinical judgment in patients with a high probability of appendicitis [2, 7]. Classically, the diagnosis of acute appendicitis is often based on a brief history, physical examination, and laboratory findings. The Alvarado score, a 10 point scoring system based on clinical signs and symptoms and a differential leukocyte count, has been applied for diagnosing appendicitis for many years [5]. Some studies have recommended an appendectomy for all patients with a score of 7 or more and observation for patients with scores of 5 or 6 [5, 7]. However, in young preverbal children, history taking and physical examination are not reliable and objective, so the score may not be applicable [8, 9]. In this situation, an objective parameter is desirable. Preoperative laboratory tests, a simple, prompt and universally available method, have been used to aid primary care clinicians in diagnosing acute appendicitis [1, 10]. However, the role of laboratory tests at different time points in diagnosing pediatric appendicitis has not been extensively explored. In this study, our intent was to establish the protocol by using different cutoff values of laboratory tests in diagnosing appendicitis based on how long the patient's symptoms were present. We further compared the diagnostic values of the protocol with the Alvarado score, and imaging examinations.

MATERIALS AND METHODS

Patient Population

A prospective study was conducted in the emergency department (ED) of a tertiary medical center in Taiwan. This study was observational without intention to influence the indications for exploration or their timing. Pediatric patients younger than 18 y of age from January 2004 to December 2006 presenting with clinically suspected acute appendicitis, as determined by an attending pediatric emergency physician (EP), were enrolled. The duration of symptoms comprised the period from the time the patients first felt ill until the time of admission. We identified the duration within 24 h as d 1, 24 to 48 h as d 2, and 48 to 72 h as d 3 in our study. Children were divided into three age groups: preschool age ($\leq$6 y), elementary school age (7–12 y), and adolescent (13–18 y). The exclusion criteria included children $<$4 y of age, patients with symptoms and signs lasting $>$3 d, and patients who were lost to follow-up at our ED or outpatient clinic. The study was approved by the institution's Human Subjects Review Committee. Informed consent was obtained from all study participants.

Study Protocol

The study protocol enrolled all patients presenting with signs and symptoms that led the pediatric EPs to conclude that acute appendicitis was the diagnostic consideration. The EPs' evaluation of the possibility of pediatric appendicitis was made based on the Alvarado score [5, 11]. Variables of this scoring system include migration of pain, anorexia, nausea/emesis, tenderness in the right iliac fossa, rebound pain, raised temperature ($>$37.3°C), leukocytosis ($>$10400/mm^3), and polymorphonuclear neutrophilia ($>$75%). A score of greater than 6 was considered as "clinically suspected acute appendicitis". All laboratory tests were obtained at the discretion of the EPs. The correlations between the different cutoff values of serum biomarkers and the Alvarado scores obtained on admission and during the duration of symptoms were analyzed statistically in all patients with suspected appendicitis. In addition, patients with a clinically equivocal diagnosis of appendicitis (Alvarado score $\leq$ 6) were admitted for clinical evaluation of the probability of appendicitis within a short observational period in the ED. The patients with equivocal presentations for appendicitis underwent diagnostic imaging examinations, including abdominopelvic computerized tomography (CT) and ultrasonography. We then further analyzed the results of diagnostic imaging examinations in these patients.

METHODS

Demographic data were recorded on a data collection sheet from the hospital charts. Our main outcome was the presence or absence of appendicitis. The presence or absence of a clinical diagnosis of appendicitis was determined by the EPs treating the patients. The ultimate diagnosis in the patients who were treated by surgical methods was based on histological examination of the excised appendix. In addition, a patient was defined as having a normal appendix when s/he was discharged without surgery from the ED and followed up by telephone interview two weeks after the index visit to confirm that appendicitis could be ruled out, or when it was found that s/he had an uninflamed appendix after having undergone an appendectomy.

Blood was sampled on admission to the hospital and analyzed for WBC count and CRP concentration. The total WBC count was measured by an automated five-part leucocyte differential count hematology analyzer (Cell-Dyn 4000R System, Abbot Laboratories, Abbot Park, IL, USA). The concentration of CRP in serum was measured by immunoturbidimetry (Beckman Coulter, Fullerton, CA). The WBC counts and CRP levels obtained from data gathered at the time of admission were also recorded on the data collection sheet. CT reports were made by senior radiologists, and abdominal ultrasonography was carried out by gastrointestinal subspecialists. We compared the diagnostic accuracy of our serum biomarkers at different cutoff values with that of the diagnostic imaging examinations (CTs and ultrasonography) for predicting appendicitis in patients with a clinically equivocal diagnosis of appendicitis.

Statistical Analysis

The methods of statistical analysis were the *t*-test, Mann-Whitney U test, and the receiver operating characteristic (ROC) curve. The selected participants were

divided into two groups: patients with normal appendices, and patients with acute appendicitis. Values are presented as mean ± standard derivation (SD). The differences between groups are presented as 95% confidence intervals (CIs). Possibility levels < 0.05 were taken as significant.

In addition, to select the cutoff values of WBC and CRP that had the best discriminatory power to distinguish acute appendicitis (both nonperforated and perforated), or perforated appendicitis from other acute abdominal diseases, the ROC curves were measured. The test characteristics of these different cutoff values, including sensitivity, specificity, area under the ROC curve (AUC), positive likelihood ratio (LR^+), and negative likelihood ratio (LR^-) were also examined. The AUC, calculated using the trapezoidal rule, was considered a global measure of the diagnostic value of the parameter. An optimal test result gives a value of 1.0, and a useless test result gives a value of 0.5. LR^+ and LR^- were calculated for the best cutoff values. The criterion value indicated the value corresponding with the highest accuracy (minimal false negative and false positive results). Statistical analyses were performed with SPSS software (ver. 12.0; SPSS, Inc., Chicago, IL).

RESULTS

Study Population

During the study period, a total of 706 children with clinically suspected acute appendicitis were enrolled. Of these children, 19 patients aged <4 y were excluded, and 26 patients whose symptoms and signs lasted for >3 d were also excluded. In addition, 67 patients who did not use our outpatient clinic for follow-up were excluded. The remaining 594 patients aged 4 to 18 y were included for further analysis. They comprised 358 males (60.3%) and 236 females (39.7%) with a mean age of 11.1 ± 4.2 y. Among the 594 patients, 213 had histologically proven nonperforated (simple) appendicitis, 93 had perforated, and 288 had normal appendices. Of those with normal appendices, 185 were treated without surgical approaches and were then discharged from our hospital, and 103 underwent normal appendectomies. The final diagnoses of patients with normal appendices included colitis, functional gastrointestinal disorders, diverticulitis, adhesion ileus, intestinal perforation, perforation of Meckel's diverticulum, mesenteric lymphadenopathy, lymphoma, and ruptured tubo-ovarian abscess. Infectious enteritis and functional gastrointestinal disorders were the most common diagnoses in patients with normal appendices. In patients with acute appendicitis, total WBC count and CRP concentration were measured in 144 cases on d 1, 135 cases on d 2, and 122 cases on d 3. In addition, 101 patients underwent abdominopelvic CT examinations, and 97 patients underwent abdominal ultrasonography for suspected appendicitis.

The majority of the 594 children in our study were in the elementary school age group (256, 43.1%), followed by the adolescent (224, 37.7%), and preschool age (114, 19.2%) groups. The time of appendicetomy in the preschool age group was commonly on d 2 and 3, while the time was more frequently on d 1 in the other two age groups. In addition, appendiceal perforation found during appendectomies was more common in the young age group (<6 y) compared with the older age group (≥6 y). The mean length of hospital stay was also longer in the young age group than that in the older age group. However, no mortality occurred in any of the cases during our study period.

Main Results

To derive a clinically useful estimate of the effect of WBC counts and CRP concentration on admission to predict pediatric appendicitis, the ROC curves of WBC counts, the CRP values and the Alvarado score were plotted. The AUCs were all greater than 0.5. The parameters selected based on the largest AUCs for discriminating acute appendicitis were taken as total WBC count on d 1, and CRP concentration on d 2 and 3. The cutoff values of the biomarkers in diagnosing acute appendicitis are listed in Table 1. Furthermore, we also calculated the best cutoff values of CRP in predicting perforated appendicitis on d 1–3, and they are

TABLE 1

The Cutoff Values of WBC Count, CRP Concentration, and the Alvarado Score ≥ 7 for Distinguishing Acute Appendicitis from Normal Appendices on D 1 to 3

Day	Parameters	Sensitivity	Specificity	LR^+	LR^-	Youden's index
1	WBC > 11000/mm^3	0.80	0.71	2.75	0.28	0.51
	Alvarado score ≥ 7	0.70	0.84	4.60	0.35	0.54
2	CRP > 25 mg/L	0.70	0.73	2.60	0.41	0.43
	Alvarado score ≥ 7	0.79	0.83	4.67	0.23	0.62
3	CRP > 89 mg/L	0.70	0.83	4.18	0.36	0.53
	Alvarado score ≥ 7	0.67	0.82	3.78	0.41	0.49

TABLE 2

The Cutoff Values of WBC Count, CRP Concentration, and the Alvarado Score ≥ 7 for Distinguishing Perforated Appendicitis from Normal Appendices on D 1–3

Day	Parameters	Sensitivity	Specificity	LR^+	LR^-	Youden's index
1	CRP > 24 mg/L	0.80	0.81	4.13	0.24	0.61
	Alvarado score ≥ 7	0.55	0.84	3.58	0.54	0.39
2	CRP > 50 mg/L	0.75	0.82	3.77	0.31	0.55
	Alvarado score ≥ 7	0.80	0.82	4.54	0.24	0.62
3	CRP > 119 mg/L	0.81	0.87	6.05	0.22	0.68
	Alvarado score ≥ 7	0.67	0.82	3.72	0.41	0.56

included in Table 2. The diagnostic accuracies (sensitivity, specificity, LR^+, LR^-, and Youden's index) of the serum biomarkers at the different cutoff values and the Alvarado score ≥7 on d 1, 2, and 3 in predicting acute appendicitis and perforated appendicitis are also presented in Table 2. The diagnostic accuracies (sensitivity, specificity, LR^+, LR^-, and Youden's index) of the serum parameters on d 1 to 3 and the Alvarado score ≥ 6 and ≥7 in predicting acute appendicitis are listed in Table 3. In addition, we further determined two cutoff points of our selected discriminators for the two diagnoses at each of the three days, and the data are presented in Table 4. The diagnostic accuracy of the serum biomarkers appeared to be as favorable as that of CT scan but appeared to be much higher than that of ultrasonography in predicting acute appendicitis (Table 5).

Furthermore, we analyzed the patients with other specific inflammatory conditions, and found colitis was the most common condition which may cause misdiagnosis and negative appendectomy in children with clinically suspected acute appendicitis. The mean score of patients with colitis, 4.34 ± 2.12, was lower than the mean score of patients with acute appendicitis (7.60 ± 1.67). In addition, the mean WBC counts and mean CRP concentration were both significantly lower in patients with colitis than in patients with acute appendicitis (WBC, $P < 0.001$; CRP, $P = 0.002$). Thus, the results indicated that colitis, the most common inflammatory condition in the non-appendicitis group, did not have an influence on the application of the Alvarado score and our selected serum parameters in children with acute appendicitis.

DISCUSSION

The main finding of this study is that a single objective serum biomarker at different time points can provide a favorable diagnostic value in the diagnosis of acute or perforated appendicitis. It is important especially when it is applied in young children. Based on the ROC analysis, we established the significant cutoff values of WBC count on d 1 and CRP concentration on d 2 and 3 in the diagnosis of acute appendicitis, and CRP on d 1–3 in the diagnosis of perforated appendicitis. In general, CRP is a good indicator of acute bacterial infection and rises earlier than WBC count. Surprisingly, WBC count had a better diagnostic value than CRP concentration on d 1 in the diagnosis of acute appendicitis but not perforated appendicitis. The reason is probably that bacterial infection is not involved significantly in the early stage of acute appendicitis.

In our study, the single serum biomarker has a predictive value as favorable as that of CT imaging and better than that of abdominal ultrasound in the diagnosis of acute or perforated appendicitis. Compared with CT

TABLE 3

The Diagnostic Accuracy of the Serum Biomarkers on D 1–3, the Alvarado Score ≥ 6, and ≥ 7 in Predicting Acute Appendicitis, and Perforated Appendicitis

Diagnostic methods	Sensitivity	Specificity	LR^+	LR^-	Youden's index
In acute appendicitis					
Serum biomarkers on day 1–3	0.74	0.75	2.94	0.35	0.49
Alvarado score ≥ 6	0.87	0.69	2.90	0.18	0.56
Alvarado score ≥ 7	0.72	0.83	4.42	0.35	0.55
In perforated appendicitis					
Serum biomarkers on day 1–3	0.79	0.83	4.38	0.23	0.62
Alvarado score ≥ 6	0.82	0.74	3.46	0.23	0.56
Alvarado score ≥ 7	0.67	0.83	4.07	0.40	0.50

TABLE 4

The Predictive Capacity of the Serum Biomarkers on D 1–3 in Acute Appendicitis with Two Cutoff Points for Each Variable

Duration	Diagnostic methods	Sensitivity	Specificity	LR^+	LR^-
In acute appendicitis					
D 1	WBC $\geq$ 7100/mm^3	1.00	0.22	1.28	0
	WBC $\geq$ 23400/mm^3	0	1.00	-	1
D 2	CRP $\geq$ 1.3 mg/L	1.00	0.23	1.27	0
	CRP $\geq$ 180 mg/L	0.09	1.00	-	0.91
D 3	CRP $\geq$ 4.6 mg/L	1.00	0.16	1.18	0
	CRP $\geq$ 214.5 mg/L	0.16	1.00	-	0.84
In perforated appendicitis					
D 1	CRP $\geq$ 16.3 mg/L	1.00	0.77	4.43	0
	CRP $\geq$ 160 mg/L	0	1.00	-	1
D 2	CRP $\geq$ 22.7 mg/L	1.00	0.69	3.20	0
	CRP $\geq$ 182.5 mg/L	0.18	1.00	-	0.82
D 3	CRP $\geq$ 62.1 mg/L	1.00	0.64	2.81	0
	CRP $\geq$ 215.5 mg/L	0.30	1.00	-	0.70

imaging, the single serum biomarker is inexpensive, objective, and readily available without the risk of radiation or the need of sedation and is more useful for the primary caregivers.

Clinically, the analyses for diagnosing appendicitis can be divided into three diagnostic test zones, one with high sensitivity, one with high specificity, and an indeterminate zone. Based on our investigation, we determined two cutoff points for each variable, which are more easily applicable in the clinical setting to ascertain at what point the variables can be ruled in and ruled out for acute appendicitis. Based on our results, we found the serum parameters to be highly valid for the diagnosis of acute appendicitis when WBC count $\geq$ 23,400/mm^3 on d 1, CRP concentration $\geq$ 180 mg/L on d 2, and CRP concentration $\geq$214.5 mg/L on d 3 were noted in children with clinically suspected appendicitis. These serum parameters were also highly valid for the exclusion of appendicitis when WBC counts were 7100/mm^3 or lower on d 1, CRP concentration was 1.3 mg/L or lower on d 2, and 4.6 mg/L or lower on d 3. For perforated appendicitis, we identified CRP concentration $\geq$160 mg/L (d 1), $\geq$182.5 mg/L (d 2), and $\geq$215.5 mg/L (d 3) to confirm perforation, and CRP concentration $<$ 16.3 mg/L (d 1), $<$ 22.7 mg/L (d 2), and $<$ 62.1 mg/L (d 3) to exclude perforation. In these situations, there may not be a need for further investigation to confirm the diagnosis in patients with clinically suspected appendicitis. In contrast, primary EPs have to pay more attention to an indeterminate zone in the ED because it is not easy for them to make decisions about patients with suspected appendicitis. Thus, they need to perform more investigations to provide further information for the diagnosis of acute appendicitis, such as diagnostic imaging examinations, short-term observation in the ED for clinical evaluation, and repeated physical and laboratory examinations. Our findings indicate that the single serum biomarker offers a rapid and helpful method to predict and discriminate pediatric appendicitis in the ED.

TABLE 5

The Diagnostic Accuracy of the Serum Biomarkers on D 1–3 and Imaging Examinations in Predicting Acute Appendicitis, and Perforated Appendicitis in Patients with the Alvarado Score ≥ 6

Diagnostic methods	Sensitivity	Specificity	LR^+	LR^-	Youden's index
In acute appendicitis					
Serum parameters on d 1–3	0.75	0.71	2.52	0.36	0.46
CT	0.78	0.70	2.59	0.32	0.48
Ultrasonography	0.47	0.74	1.80	0.72	0.21
P value	$<$0.001	0.852			0.001
In perforated appendicitis					
Serum parameters on d 1–3	0.78	0.83	4.45	0.27	0.61
CT	0.81	0.70	2.70	0.27	0.51
Ultrasonography	0.64	0.74	2.46	0.49	0.38
P value	0.485	0.721			0.389

CONCLUSION

A single serum biomarker at different time points has a diagnostic value that is as favorable as that of Alvarado score and CT imaging and better than that of ultrasonography in predicting pediatric acute or perforated appendicitis. It is inexpensive, objective, and readily available without the risk of radiation or the need of sedation and is useful for primary caregiver.

REFERENCES

1. Cappendijk VC, Hazebroek FW. The impact of diagnostic delay on the course of acute appendicitis. Arch Dis Child 2000;83:64.
2. Wu HP, Fu YC. Application with repeated serum biomarkers in pediatric appendicitis in clinical surgery. Pediatr Surg Int 2010; 26:161.
3. El-Metwally A, Halder S, Thompson D, et al. Predictors of abdominal pain in schoolchildren: A 4-year population-based prospective study. Arch Dis Child 2007;92:1094.
4. Wu HP, Chang CF, Lin CY. Predictive inflammatory parameters in the diagnosis of acute appendicitis in children. Acta Paediatr Taiwan 2003;44:227.
5. Douglas CD, Macpherson NE, Davidso PM, et al. Randomized controlled trial of ultrasonography in diagnosis of acute appendicitis, incorporating the Alvarado score. BMJ 2000; 321:919.
6. Grönroos P, Huhtinen H, Grönroos JM. Normal leukocyte count and C-reactive protein value do not effectively exclude acute appendicitis in children. Dis Colon Rectum 2009; 52:1028.
7. Bundy DG, Byerley JS, Liles EA, et al. Does this child have appendicitis? JAMA 2007;298:438.
8. Bond GR, Tully SB, Chan LS, et al. Use of the MANTRELS score in childhood appendicitis: A prospective study of 187 children with abdominal pain. Ann Emerg Med 1990;19:1014.
9. Owen TD, Williams H, Stiff G, et al. Evaluation of the Alvarado score in acute appendicitis. J R Soc Med 1992;85:87.
10. Clyne B, Olshaker JS. The C-reactive protein. J Emerg Med 1999;17:1019.
11. Alvarado A. A practical score for the early diagnosis of acute appendicitis. Ann Emerg Med 1986;15:557.