Significant change between primary and repeated serum laboratory tests at different time points in pediatric appendicitis

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Objective **The aim of the work is to determine whether the change between primary and repeated serum inflammatory markers measured 8–12 h later may improve diagnostic accuracy in pediatric appendicitis.**

Methods **The study group comprised 258 pediatric patients with clinically suspected appendicitis admitted to the pediatric emergency department from 2005 to 2007. The significant changes in serum parameters between primary and repeated examinations were selected as the discriminating variables. The receiver operating characteristic curves were used to determine the cut-off values of the changes between two examinations in predicting appendicitis.**

Results **Receiver operating characteristic analysis showed that the cut-off values for the change in total neutrophils (3.0%) on the first day after the onset of symptoms (day 1), the changes in C-reactive protein concentration (4.5 mg/l) and in bands (1%) on day 2, and the change in C-reactive protein concentration (15.0 mg/l) on day 3 were significant serum parameters in predicting pediatric appendicitis.**

Conclusion **Repeated serum laboratory tests at different time points during the progression of acute appendicitis may be helpful in predicting pediatric appendicitis in the pediatric emergency department.** ***European Journal of Emergency Medicine*** **00:000–000** © **2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Acute appendicitis is one of the most common surgical emergencies in the pediatric emergency department (PED) [1,2]. However, the diagnosis of appendicitis is problematic in children because many cases may present with signs and symptoms mimicking other conditions related to acute abdominal pain [1–5]. The definitive diagnosis of appendicitis is made in only 50–70% of children at the time of initial assessment of acute abdominal pain in the PEDs [6]. The clinical challenge for primary clinicians is to diagnose appendicitis early to prevent perforation of an appendix, while minimizing the number of negative appendectomies that are performed. Although abdominal ultrasonography and computed tomographic scanning are helpful in diagnosing acute abdomen preoperatively [1–5,7], these modalities are not always readily available in all primary healthcare settings. Furthermore, it has been argued that findings of imaging studies should not supersede clinical judgment in patients with a high probability of appendicitis. In addition, in about 10–15% of cases with suspected appendicitis, radiological studies such as computed tomography or ultrasound, performed to rule out appendicitis, may show a questionable finding to suggest this diagnosis [8,9].

Analyses of serum laboratory tests, including white blood cell count, total neutrophil counts, manual band counts, and C-reactive protein (CRP), are relatively rapid, cheap, and universally available, and have been used as indicators to evaluate inflammation related to the abdomen such as acute appendicitis [10–12]. However, the role of repeated laboratory tests at different time points during the process of appendicitis has not been extensively explored in detail. In this study, we aim to study whether repeated laboratory examination can provide further aids in diagnosing pediatric appendicitis and to determine the extent to which the change between primary and repeated examinations can serve as useful parameters in predicting appendicitis.

Methods

Patient population

We prospectively recruited consecutive pediatric patients less than 18 years of age with suspected acute appendicitis in the PED at a medical center in central

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Taiwan from 2005 to 2007. 'Clinically suspected acute appendicitis' was reserved for cases with clinical symptoms and signs that included abdominal pain, anorexia, nausea, vomiting, pyrexia, migration of pain, and tenderness over the right lower quadrant of the abdomen [13]. Patients with suspected appendicitis in whom repeated laboratory examinations were performed 8–12 h later were included in our study, but those who were treated by nonsurgical methods were excluded. The ultimate diagnosis of the patients who underwent surgical approaches was based on histopathological examination of the excised appendix. In addition, a patient was defined as having a normal appendix when symptoms and signs in a nonoperative patient subsided and the patient was discharged, and when follow-up at the outpatient clinic 2 weeks later confirmed that the diagnosis of appendicitis could be ruled out. A normal appendix was also defined when an uninflamed appendix was found in a patient who had undergone appendectomy with intent-to-treat acute appendicitis (normal appendectomy).

Methods

In the hospital charts, the following data were recorded on admission: age, sex, body temperature, time of onset of symptoms, and time of admission. We identified the duration within 24 h as day 1, 24–48 h as day 2, and 48–72 h as day 3. On admission, the blood samples were obtained from all patients for further analysis. Repeated laboratory examination was performed in patients with clinically suspected appendicitis after 8–12 h of observation. The correlation between repeated laboratory examination and the period recorded from the onset of symptoms to admission on days 1–3 was analyzed statistically. The total white blood cell and neutrophil counts were measured using an automated five-part leukocyte differential count hematology analyzer (Cell-Dyn 4000R System; Abbot Laboratories, Abbot Park, Illinois, USA). The nonsegmented bands were counted manually by counting 100 consecutive cells in a peripheral blood film. The concentration of CRP in the serum was measured by immunoturbidimetry (Beckman Coulter, Fullerton, California, USA).

Statistical analysis

Statistical analysis was carried out using the *t*-test, the Mann–Whitney *U*-test, and the receiver operating characteristic (ROC) curve. The difference between groups was presented as 95% confidence intervals. Possibility levels of less than 0.05 were taken as significant. The informative value of each biological marker was determined and the cut-off points were defined by ROC analysis. The area under the ROC curve (AUC), calculated using the trapezoidal rule, was considered a global measure of the diagnostic value of the parameter. Positive and negative likelihood ratios (LR^+ = ratio of the fraction of true positives and false positives, and negative LR^- = ratio of the fraction of false negatives and true negatives, respectively) were calculated for the best cut-off values. Statistical analyses were performed using SPSS software (version 11.0; SPSS Inc., Chicago, Illinois, USA).

Results

During our study period, a total of 258 patients with suspected appendicitis were admitted to the hospital and underwent repeated laboratory examinations 8–12 h later. The patient group comprised 156 boys (60%) and 102 girls (40%) with a mean age of 11.5 years (range, 1–18 years). Of these, 166 had histologically proven acute appendicitis and 92 had normal appendices. Of the clinically diagnosed cases of acute appendicitis on day 1, 70/111 (63.1%) had histologically proven acute appendicitis; on day 2, 56/75 (74.7%); and on day 3, 40/72 (55.6%). If the laboratory parameters had been applied to these cases to rule out surgery on day 1 with a Δ neutrophil cut-off of more than 3.0%, only 53/111 would have had surgery; of those not having surgery, none would have had appendicitis. On day 2, with a Δ band cut-off of more than 1%, only 36/75 would have had surgery; of those not having surgery, none would have had appendicitis. With a cut-off of CRP > 4.5 mg/l, 51/75 would have had surgery, and of those not having surgery, none would have had appendicitis. With a cut-off of a Δ band greater than 1.0% and CRP > 4.5 mg/l only 36/75 would have had surgery, and of those not having surgery none would have had appendicitis. On day 3, with a cut-off of CRP > 15.0 mg/l only 31/72 would have had surgery, and of those not having surgery, none would have had appendicitis.

The mean differences in the changes in serum laboratory examinations between patients with appendicitis and normal appendices were analyzed based on the first 3 days after onset of symptoms (Table 1). On day 1, the change in total neutrophils (Δ neutrophils) was statistically significantly higher in patients with appendicitis than those with normal appendices ($P < 0.05$). On day 2, the changes in the manual band counts (Δ bands) and in the CRP concentration (Δ CRP) were both significantly higher in patients with appendicitis ($P < 0.05$). On day 3, only Δ CRP was statistically significant ($P < 0.005$).

Furthermore, the abilities of the changes between the primary and repeated laboratory examinations in our selected significant parameters (Δ neutrophils on day1, Δ bands on day 2, and Δ CRP on days 2 and 3) in predicting appendicitis were further analyzed using the ROC curves (Fig. 1). The AUCs of our selected parameters were all greater than 0.5. The diagnostic accuracies (sensitivity, specificity, LR^+, and LR^-) of the different cut-off changes in the serum parameters in predicting appendicitis on days 1–3 were determined and are shown in Table 2. The best cut-off value of Δ neutrophils on day 1 was 3.0%, the cut-off value of Δ bands on day 2 was 1.0%, and the cut-off values of Δ CRP were 4.5 mg/l on

day 2 and 15.0 mg/l on day 3 in distinguishing acute appendicitis from other acute abdominal diseases in children. In addition, we further determined two cut-off points of our selected discriminators to 'rule in' or 'rule out' acute appendicitis at each of the 3 days in Table 3.

Table 1 Mean differences in the changes in serum laboratory examinations between acute appendicitis and normal appendices

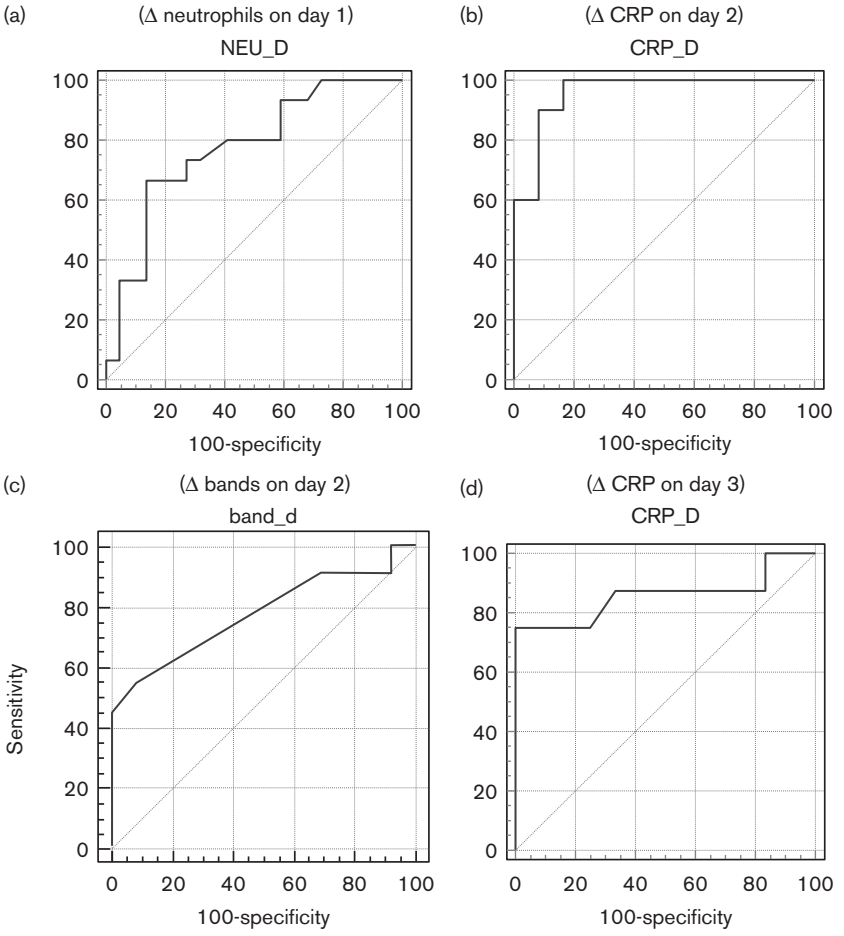
Day	Mean difference (95% CI)	*P* value
Day 1		
Δ WBC (/mm^3)	−1715.61 (−3818.16 to 386.95)	0.107
Δ Neutrophils (%)	9.8 (1.90–17.74)	0.017
Δ Manual bands (%)	0.02 (−1.59 to 1.56)	0.981
Δ CRP (mg/l)	0.30 (−1.26 to 1.87)	0.696
Day 2		
Δ WBC (/mm^3)	3610.89 (−865.11 to 8086.79)	0.168
Δ Neutrophils (%)	9. 73 (−0.02 to 19.49)	0.051
Δ Manual bands (%)	7.62 (0.74–14.49)	0.032
Δ CRP (mg/l)	5.52 (0. 95–10.09)	0.023
Day 3		
Δ WBC (/mm^3)	−1245.19 (−5216.19 to 2725.80)	0.490
Δ Neutrophils (%)	55.76 (89.77–201.28)	0.432
Δ Manual bands (%)	3.30 (−2.86 to 9.46)	0.276
Δ CRP (mg/l)	5.68 (0.36–11.30)	0.048

CRP, C-reactive protein; WBC, white blood cells; Δ, the change in serum parameters between two examinations.

Discussion

Abdominal pain is one of the most common presenting symptoms in children brought to PED [13,14]. The causes of abdominal pain in children range from simple etiologies to potentially catastrophic ones. Distinguishing appendicitis from other disorders is difficult, particularly in young children [2,13]. However, early diagnosis of appendicitis can not only prevent perforation, abscess formation, and postoperative complications, but also decrease cost by decreasing hospitalization time [4,12]. Classically, the diagnosis of acute appendicitis is often based on a brief history and physical examination such as migration of abdominal pain, anorexia, nausea, vomiting, abdominal rebounded pain, and tenderness over the right lower quadrant of the abdomen. Nevertheless, in some cases, for example, in young, preverbal children, history taking is difficult and physical examination findings are

Fig. 1



Receiver operating characteristic curves for the changes in the total neutrophil count on day 1 (a), C-reactive protein (CRP) (b), band count (c) on day 2, and CRP on day 3 (d) between primary and repeated examinations in distinguishing pediatric appendicitis and normal appendices.

Table 2 Sensitivity, specificity, LR^+, and LR^- at the cut-off values of the changes between primary and repeated laboratory examinations in distinguishing pediatric appendicitis from normal appendices

Duration	Parameters	Sensitivity	Specificity	LR^+	LR^-	AUC (95% CI)
Day 1	Δ Neutrophils >3.0%	0.67	0.87	4.89	0.39	0.77 (0.65–0.90)
Day 2	Δ CRP >4.5 mg/l	0.90	0.92	10.8	0.11	0.95 (0.87–0.99)
Day 2	Δ Bands >1.0%	0.55	1.00	–	0.47	0.77 (0.58–0.92)
Day 3	Δ CRP >15.0 mg/l	0.75	1.00	–	0.25	0.86 (0.75–0.97)

AUC, the areas under the receiver operating characteristic curve; CI, confidence interval; LR^-, negative likelihood ratio; LR^+, positive likelihood ratio; Δ, the change in serum parameters between two examinations.

Table 3 The predicting capacity in acute appendicitis with two cut-off points for each variable on days 1–3

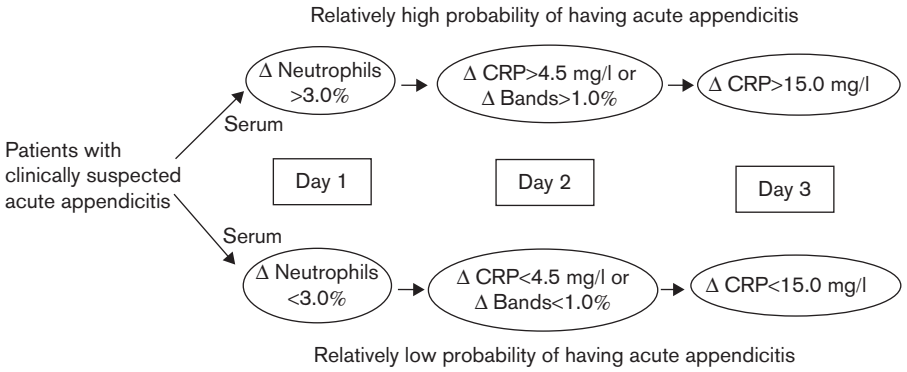
Parameters	Cut-off values	Sensitivity	Specificity	LR^+	LR^-	AUC (95% CI)
Day 1						
Δ Neutrophils	> −9.7%	1.00	0.27	1.37	0	0.77 (0.65–0.90)
	>24.8%	0.07	1.00	–	0.93	0.77 (0.65–0.90)
Day 2						
Δ CRP	>2.32 mg/l	1.00	0.80	5.00	0	0.95 (0.87–0.99)
	>16.9 mg/l	0.63	1.00	–	0.37	0.95 (0.87–0.99
Δ Bands	> −29%	1.00	0.08	1.08	0	0.77 (0.58–0.92)
	>1.0%	0.55	1.00	–	0.47	0.77 (0.58–0.92)
Day 3						
Δ CRP	> −11.6 mg/l	1.00	0.24	1.31	0	0.86 (0.75–0.97)
	>16.9 mg/l	0.82	1.00	–	0.18	0.86 (0.75–0.97)

AUC, the areas under the receiver operating characteristic curve; CI, confidence interval; LR^-, negative likelihood ratio; LR^+, positive likelihood ratio; Δ, the change in serum parameters between two examinations.

easily equivocal [1,12–14]. Clinicians may be uncertain how to interpret children with equivocal clinical presentations of acute appendicitis. Imaging techniques have been shown to be particularly accurate for diagnosing appendicitis; however, they are not readily available in all primary healthcare settings, and their potential risks for exposure to ionizing radiation may result in increased healthcare costs. These concerns have led to renewed interest in clinical scoring systems and laboratory tests to better diagnose appendicitis.

In this study, in the 258 patients with clinically suspected appendicitis, 166 (64.3%) had acute appendicitis and 92 (35.7%) were in the normal appendix group. The diagnostic rate based on the initial clinical diagnosis of primary clinicians was 64.3% but the diagnostic rate based on our repeated laboratory tests may reach 83.3% on days 1–3. The accurate diagnostic rate of repeated laboratory tests in predicting appendicitis seemed to be higher than the initial clinical diagnosis in the emergency department. In addition, our data demonstrated that total neutrophils could be considered as a useful inflammatory parameter on day 1, and the change between primary and repeated examinations could significantly increase the diagnostic accuracy in early appendicitis. The cut-off point of Δ neutrophils on day 1 was determined on the basis of the result of ROC analysis. Clinically, once Δ neutrophil is greater than 3.0% on day 1, the probability of acute appendicitis will increase. On day 2, Δ bands and Δ CRP were important predictors in diagnosing appendicitis, but on day 3, only CRP appeared to be the most important predictor in diagnosing appendicitis in our study. We also determined the best cut-off values of Δ bands and Δ CRP on the 2 days by analyzing ROC curves. Clinically, once the Δ band is greater than 1%, Δ CRP is greater than 4.5 mg/l on day 2, and Δ CRP is greater than 15.0 mg/l on day 3, the probability of acute appendicitis will significantly increase. The larger AUC indicates a higher diagnostic capacity. In our study, we found that the AUCs of Δ CRP were both favorable on days 2 and 3. This may indicate that Δ CRP could serve as a strong predictor in diagnosing appendicitis on days 2 and 3. On the basis of kinetic studies, severe inflammation may prime and activate neutrophils within 3–6 h after injury, and bacterial infection may prime neutrophils with an optimum incubation time of 25 min [15–17], but CRP, an acute-phase protein, may show an increase of serial serum levels after 12–24 h of severe inflammation [11,12]. These results may be compatible with our findings, which showed a significant change in total neutrophils in early stage of the process in acute appendicitis and a significant change in the CRP concentration in the latter stage. In addition, clinically, once the clinical diagnosis of acute appendicitis is made, consequent appendectomy will be indicated [18–21]. We suggest that children with suspected appendicitis should be subjected to careful clinical evaluation. To illustrate how to apply the findings clinically, we have established a decision tree for primary clinicians to aid decision-making in pediatric patients with clinically suspected acute appendicitis (Fig. 2). Indeed, we believe that repeated examination of the serum laboratory parameters can aid primary emergency physicians in integrating the indications of surgical intervention in pediatric patients with suspected appendicitis.

Fig. 2



A decision tree for primary clinicians to aid decision-making in pediatric patients with clinically suspected acute appendicitis.

In conclusion, significant changes between primary and repeated serum laboratory tests during in-hospital observation may help establish the diagnosis of acute appendicitis in children. We propose the addition of repeated serum biomarkers as a helpful method in pediatric patients suspected of having acute appendicitis.

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Conflicts of interest

There are no conflicts of interest.

References

1 Lin KC, Wu HP, Huang CY, Lin CY, Chang CF. Discriminant analysis of serum inflammatory biomarkers which differentiate pediatric appendicitis from other acute abdominal diseases. *Acta Paediatr Taiwan* 2007; **48**: 125–130.
2 Tseng YC, Lee MS, Chang YJ, Wu HP. Acute abdomen in pediatric patients admitted to the pediatric emergency department. *Pediatr Neonatol* 2008; **49**:126–134.
3 Denizbasi A, Unluer EE. The role of the emergency medicine resident using the Alvarado score in the diagnosis of acute appendicitis compared with the general surgery resident. *Eur J Emerg Med* 2003; **10**:296–301.
4 Douglas CD, Macpherson NE, Davidson PM, Gani JS. Randomised controlled trial of ultrasonography in diagnosis of acute appendicitis, incorporating the Alvarado score. *BMJ* 2000; **321**:919–922.
5 Nance ML, Adamson WT, Hedrick HL. Appendicitis in the young child: a continuing diagnostic challenge. *Pediatr Emerg Care* 2000; **16**:160–162.
6 Wyer PC. Decision rule can help identify children at lower risk for appendicitis. *J Pediatr* 2006; **148**:142–143.
7 Toorenvliet BR, Wiersma F, Bakker RF, Merkus JW, Breslau PJ, Hamming JF. Routine ultrasound and limited computed tomography for the diagnosis of acute appendicitis. *World J Surg* 2010; **34**:2278–2285.
8 Daly CP, Cohan RH, Francis IR, Caoili EM, Ellis JH, Nan B. Incidence of acute appendicitis in patients with equivocal CT findings. *Am J Roentgenol* 2005; **184**:1813–1820.
9 Funaki B, Grosskreutz SR, Funaki CN. Using unenhanced helical CT with enteric contrast material for suspected appendicitis in patients treated at a community hospital. *Am J Roentgenol* 1998; **171**:997–1001.
10 Kwan KY, Nager AL. Diagnosing pediatric appendicitis: usefulness of laboratory markers. *Am J Emerg Med* 2010; **28**:1009–1015.
11 Allister L, Bachur R, Glickman J, Horwitz B. Serum markers in acute appendicitis. *J Surg Res* 2011; **168**:70–75.
12 Wu HP, Lin CY, Chang CF, Chang YJ, Huang CY. Predictive value of C-reactive protein at different cutoff levels in acute appendicitis. *Am J Emerg Med* 2005; **23**:60–66.
13 Monneuse O, Abdalla S, Pilleul F, Hervieu V, Gruner L, Tissot E, *et al.* Pain as the only consistent sign of acute appendicitis: lack of inflammatory signs does not exclude the diagnosis. *World J Surg* 2010; **34**:210–215.
14 Fox JC, Solley M, Anderson CL, Zlidenny A, Lahham S, Maasumi K. Prospective evaluation of emergency physician performed bedside ultrasound to detect acute appendicitis. *Eur J Emerg Med* 2008; **15**:80–85.
15 Botha AJ, Moore FA, Moore EE, Kim FJ, Banerjee A, Peterson VM. Postinjury neutrophil priming and activation: an early vulnerable window. *Surgery* 1995; **118**:358–364.
16 Van Leeuwen HJ, Van Der Tol M, Van Strijp JA, Verhoef J, van Kessel KP. The role of tumour necrosis factor in the kinetics of lipopolysaccharide-mediated neutrophil priming in whole blood. *Clin Exp Immunol* 2005; **140**:65–72.
17 Brazil TJ, Dagleish MP, McGorum BC, Dixon PM, Haslett C, Chilvers ER. Kinetics of pulmonary neutrophil recruitment and clearance in a natural and spontaneously resolving model of airway inflammation. *Clin Exp Allergy* 2005; **35**:854–865.
18 Jen HC, Shew SB. Laparoscopic versus open appendectomy in children: outcomes comparison based on a statewide analysis. *J Surg Res* 2010; **161**:13–17.
19 Abarbanell AM. Trends in pediatric appendectomy outcomes. *J Surg Res* 2010; **161**:233–234.
20 Cavuşoğlu YH, Erdoğan D, Karaman A, Aslan MK, Karaman I, Tütün OC. Do not rush into operating and just observe actively if you are not sure about the diagnosis of appendicitis. *Pediatr Surg Int* 2009; **25**:277–282.
21 Kutasy B, Hunziker M, Laxamanadass G, Puri P. Laparoscopic appendectomy is associated with lower morbidity in extremely obese children. *Pediatr Surg Int* 2011; **27**:533–536.