Could C-reactive protein be a potential biomarker of complicated acute appendicitis?
C反應蛋白能否成為急性闌尾炎併發症的準生物標誌？

CY Lai 黎鎮宇, YK Leung 梁玉騏, CA Graham 簡家麓

Introduction: The purpose of this study is to evaluate the diagnostic value of C-reactive protein (CRP) to identify complicated appendicitis (defined as perforation, abscess or gangrenous appendicitis) versus simple appendicitis. Methods: A retrospective study was performed in the emergency department (ED) of Prince of Wales Hospital in the New Territories of Hong Kong. The clinical records of patients admitted to the surgical wards via the ED over 17 months were reviewed by a research assistant. We included all patients aged >13 years who had a provisional diagnosis of “acute appendicitis” or “right lower quadrant pain”. We excluded patients with pregnancy, previous appendectomy, incomplete medical records or no CRP levels determined before operation and those who discharged themselves from hospital against medical advice after admission. The outcome of this study was the CRP level in relation to the presence of complicated appendicitis. ROC curve analysis was employed to identify the optimum cut-off level of CRP which had the best sensitivity and specificity to identify complicated appendicitis compared to simple appendicitis. Results: Forty-two patients were diagnosed to have acute appendicitis, 31 of whom had complicated appendicitis. CRP level was higher in the group of complicated appendicitis (p<0.05). We identified an optimum cut-off value for CRP of 40.1 mg/L to discriminate between complicated and non-complicated appendicitis. The sensitivity and specificity of CRP to identify complicated appendicitis were 71.0% and 100% respectively at this cut-off level. Conclusion: High CRP levels could possibly predict the diagnosis of complicated appendicitis and facilitate more appropriate surgical care. This finding needs to be confirmed in prospective and larger multicentre studies. (Hong Kong J. Emerg. Med. 2014;21:354-360)

簡介：本研究的主要目的在於評鑑 C反應蛋白在急性闌尾炎併發症（定義為穿孔、膿腫或壞疽）診斷上的價值。方法：這項回溯性研究於香港沙田威爾斯親王醫院急症室進行，由一位研究員負責翻查17個月內從急症室收入外科的入院紀錄。我們收集所有13歲以上被臨時診斷為患有「急性闌尾炎」或「右下腹疼痛」的病人；而正在懷孕、曾經做過闌尾切除術、欠缺完整醫療紀錄、欠缺血液C反應蛋白檢驗報告及不遵從醫生勸告自行出院的病人則會被排除。研究結果定為C反應蛋白水平與闌尾炎併發症出現的關係；而接收者操作特徵曲線（ROC curve）分析則用作尋找擁有最佳敏感度和特異度的C反應蛋白臨界值，以協助診斷併發症闌尾炎。結果：42位病人被診斷為患有急性闌尾炎，當中有31位出現併發症。併發症闌尾炎組別的病人的C反應蛋白水平較高（p 值少於0.05）。我們發現C反應蛋白在闌尾炎併發症的診斷臨界值為40.1 mg/L；而此臨界值的敏感度和特異度分別為71.0% 和

Correspondence to:
Lai Chun Yu, MBBS, MCEM
Prince of Wales Hospital, Accident and Emergency Department, 30-32 Ngan Shing Street, Shatin, N.T., Hong Kong
Email: cosmopolitanlaiy@yahoo.com.hk

Colin A Graham, MD, FHKCEM. FHKAM(Emergency Medicine)

Prince of Wales Hospital, Accident and Emergency Medicine Academic Unit, The Chinese University of Hong Kong, 2/F, Main Clinical Block and Trauma Centre, 30-32 Ngan Shing Street, Shatin, N.T., Hong Kong
Leung Yuk Ki, BSc, PhD
Colin A Graham, MD, FHKCEM. FHKAM(Emergency Medicine)
Introduction

Acute appendicitis is one of the commonest urgent abdominal conditions encountered in emergency departments (ED). Prompt diagnosis of this disease entity remains challenging for most emergency physicians as the early symptoms are vague, especially for the elderly, and delays in treatment can lead to devastating consequences.1-3 Nowadays, accurate detection of appendicitis is possible due to the development of computed tomography (CT);4 however, this imaging modality is usually not immediately available, especially at night. A biomarker which can predict which patient requires immediate surgical intervention may be important to reduce mortality and morbidity secondary to inadequate or delayed diagnosis.

Although C-reactive protein (CRP) is well-known to be a non-specific marker of inflammation, recently it has been shown to have some value in the diagnosis of appendicitis, and in particular, complicated appendicitis5-9 (defined as perforation, abscess or gangrenous appendicitis). Laboratory testing for CRP level is readily available round the clock in Prince of Wales Hospital. Test results, which are obtainable within 1-2 hours, can assist our decision making in the ED.

The aim of this study was to determine if the CRP level, measured on arrival at the ED, could be a useful biomarker to predict complicated appendicitis compared to simple (uncomplicated) appendicitis.

Methods

Study design
This investigation was a retrospective study carried out in the ED of Prince of Wales Hospital (PWH) in the New Territories of Hong Kong. Prince of Wales Hospital is the teaching hospital of the Chinese University of Hong Kong and serves the population of Shatin (around 600 000 people). It is the tertiary referral hospital and neurosciences and trauma centre for the Eastern New Territories of Hong Kong, which has a population of 1.5 million people. The ED has an annual census of 150 000.

Inclusion criteria
The clinical records of patients who were admitted to the general surgical wards via the ED from 14 December 2011 to 14 May 2013 were reviewed by a research assistant. We included all the patients who had a provisional diagnosis of "acute appendicitis", "suspected acute appendicitis" or "right lower quadrant pain for investigations" as documented on the ED notes, irrespective of race, gender, triage category and past medical illness. Age was limited to >13 years as patients younger than this were admitted to the paediatric surgery wards and thus were not listed on the general surgical admission list.

Exclusion criteria
We excluded patients with pregnancy, previous appendicectomy or incomplete medical records and those who had no blood taken for CRP level before operation and those who discharged themselves from hospital against medical advice (DAMA). For those patients who were discharged from the surgical wards without undergoing appendicectomy, we assumed that they did not have appendicitis if they were not readmitted and/or did not undergo appendicectomy within 14 days of discharge.

Definitions
Complicated appendicitis was defined as being present when there was evidence of gangrenous changes,
abscess formation or appendix perforation on the histopathology reports. Operative findings written by the operating surgeon in the clinical records were also taken into consideration to make the diagnosis of complicated appendicitis. Simple appendicitis was defined as appendicitis diagnosed at operation and/or histopathologically which did not meet the criteria for complicated appendicitis.

Data collection
We traced the progress of recruited patients through the electronic patient record (ePR) after admission. We recorded patients' characteristics, CRP level, time to operation and final discharge diagnosis for all patients. Our laboratory was utilising Roche Cobas C8000 modular analyser series for CRP level measurement in our study. CRP level determination was done by immunoturbidimetric assay and the cut-off was suggested to be 9.9 mg/L by the manufacturer. Patient who were suspected or confirmed by imaging to have acute appendicitis would undergo open or laparoscopic appendicectomy. We reviewed the operative and histopathology reports of the excised appendices whenever they were available. We followed the definitions as listed above to divide the patients into complicated or simple appendicitis group.

Outcome of interest
The primary outcome of this study was the CRP level in relation to the presence of complicated appendicitis. Secondary outcomes included the optimum cut-off level for CRP that was close to left upper corner of ROC curve with maximal specificity for the diagnosis of complicated appendicitis.

Sample size calculation
Our sample size was estimated using MedCalc (v12.4.0.0, MedCalc Software bvba, Belgium) under the section of Sample size calculation: Area under ROC curve (http://www.medcalc.org/manual/sampling_ROC1.php). Let: Type I error (Alpha) = 0.05, Type II error (Beta, 1-Power)=0.2, Area under ROC curve=0.8, and Null hypothesis value=0.5. Using data from a large American study, let: Ratio of sample sizes in negative/positive groups: 2. Therefore, the number of positive and negative cases required were 10 and 20 respectively and the total number of patients required were 30.

Data analysis
Laboratory parameters such as CRP were presented in medians with interquartile ranges as they were not normally distributed. The Mann-Whitney U-Test was used to compare the CRP levels of complicated appendicitis with those of simple appendicitis. Multivariate logistic regression analysis using the stepwise backward method was performed to evaluate independent predictors of complicated appendicitis. Statistical tests were performed by IBM SPSS Statistics (IBM SPSS Statistics v20.0, NY, USA). Receiver operating characteristic (ROC) curve analysis using MedCalc (v12.4.0.0, MedCalc Software bvba, Belgium) was employed to determine the association of complicated appendicitis with CRP and to calculate the optimum cut-off level. All tests were considered to be significant at p<0.05.

Institutional review board approval
This study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee on 22 February 2013 (CREC Ref. No: CRE-2013.034).

Results
Figure 1 details the number of patients in the study according to inclusion and exclusion criteria. Acute appendicitis was diagnosed in 42 patients. Basic patient characteristics are shown in Table 1. The median of CRP in the complicated appendicitis group was higher than that of the simple appendicitis group.

ROC curve was drawn and shown in Figure 2. The area under the ROC curve (AUC) for CRP for complicated appendicitis compared to simple appendicitis was 0.864 (95% confidence interval [CI]) =0.722 to 0.950, p<0.001). A point on ROC curve, in which CRP was 40.1 mg/L, had optimum diagnostic performance with maximal specificity and it was selected as a new cut-off. At cut-off point of 40.1 mg/L, the sensitivity, specificity, positive predictive value and negative predictive value were 71.0% (95% CI=53.4% to 83.9%), 100.0% (95% CI=74.1% to 100.0%), 100.0 (95% CI=85.1 to 100.0%) and 55.0% (95% CI=34.2% to 74.2%) respectively
Number of surgical admissions identified within the period with provisional diagnosis of acute appendicitis or right lower quadrant pain for investigations = 299

Number of subjects included
N = 122

Number of laparoscopic or open appendicectomies = 50

Number of acute appendicitis (Operative or histological diagnosis) = 42

Number of subjects without acute appendicitis = 80

Number of simple (uncomplicated) appendicitis = 11

Complicated appendicitis = 31
Gangrenous changes (20)
Abscess formations (15)
Perforations (16)
These complications were not mutually exclusive.

DAMA = discharge against medical advice

**Figure 1.** Study inclusion and exclusion flowchart.

**Table 1.** Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Complicated group</th>
<th>Simple group</th>
<th>Non-appendicitis group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>31</td>
<td>11</td>
<td>80</td>
<td>122</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>58.32±20.10</td>
<td>39.09±15.44</td>
<td>37.42±13.87</td>
<td>42.90±18.10</td>
</tr>
<tr>
<td>Median</td>
<td>64</td>
<td>35</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17/31 (54.8%)</td>
<td>5/11 (45.5%)</td>
<td>25/80 (31.25%)</td>
<td>47/122 (38.52%)</td>
</tr>
<tr>
<td>Female</td>
<td>14/31 (45.2%)</td>
<td>6/11 (54.5%)</td>
<td>55/80 (68.75%)</td>
<td>75/122 (61.48%)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>72.0* (88)</td>
<td>11.0* (30)</td>
<td>11.5 (36)</td>
<td>21.5 (65)</td>
</tr>
</tbody>
</table>

*Mann-Whitney U-Test: Complicated Group vs Simple Group; p<0.001.
CRP=C-reactive protein; SD=standard deviation; IQR=interquartile range
(Table 2). In Prince of Wales Hospital, a CRP level of 9.9 mg/L was suggested by the diagnostic utility manufacturer to be the upper limit of normal. The sensitivity, specificity, positive predictive value and negative predictive value at this cut-off point (9.9 mg/L) were 90.3% (95% CI=75.1% to 96.7%), 45.5% (95% CI=21.3% to 72.0%), 82.4% (95% CI=66.5% to 91.7%) and 62.5% (95% CI=30.6% to 86.3%) respectively.

Multivariate logistic regression (Table 3) was performed to investigate the predictive performance of age, gender, white cell count and CRP. Age, gender and white cell count were all not statistically significant but CRP had an odds ratio of 1.058 (95% CI=1.008 to 1.111, p=0.022) for complicated appendicitis compared to simple appendicitis. The means of time to operation of complicated and simple groups were 1950±1333 minutes and 1916±817 minutes respectively and they were not statistically different.

**Discussion**

The results of this study suggested that an elevated level of CRP taken on admission to the ED could help the clinician to distinguish between complicated appendicitis and simple appendicitis. CRP is well-known to be a non-specific inflammatory marker as its level is increased in many conditions. The suggested cut-off level of CRP in PWH (9.9 mg/L) may be too low to identify complicated appendicitis. CRP at the higher cut-off level of 40.1 mg/L had good specificity

---

**Table 2.** Sensitivity and specificity of diagnosis of complicated appendicitis by C-reactive protein (CRP) at different cut-off levels

<table>
<thead>
<tr>
<th>CRP</th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
<th>+LR</th>
<th>-LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&gt;9.9 mg/L)</td>
<td>90.3% (75.1% to 96.7%)</td>
<td>45.5% (21.3% to 72.0%)</td>
<td>82.4% (66.5% to 91.7%)</td>
<td>62.5% (30.6% to 86.3%)</td>
<td>1.656</td>
<td>0.213</td>
</tr>
<tr>
<td>(&gt;40.1 mg/L)</td>
<td>71.0% (53.4% to 83.9%)</td>
<td>100.0% (74.1% to 100.0%)</td>
<td>100.0% (85.1% to 100.0%)</td>
<td>55.0% (34.2% to 74.2%)</td>
<td>&gt;10</td>
<td>0.290</td>
</tr>
</tbody>
</table>

*Remarks: PPV=positive predictive value; NPV=negative predictive value; +LR=positive likelihood ratio, -LR=negative likelihood ratio

**Table 3.** Results of multivariate analysis

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio of complicated appendicitis</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.041</td>
<td>0.974-1.112</td>
<td>0.240</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.087</td>
<td>0.316-13.802</td>
<td>0.445</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>1.058</td>
<td>1.008-1.111</td>
<td>0.022*</td>
</tr>
<tr>
<td>White blood cell count</td>
<td>1.177</td>
<td>0.896-1.547</td>
<td>0.241</td>
</tr>
</tbody>
</table>

*Statistically significant, i.e. p<0.05
(100%, 95% CI=74.1% to 100.0%) and PPV (100%, 95% CI=85.1% to 100%), this could potentially be used to rule in complicated appendicitis in clinically compatible cases to facilitate earlier surgical intervention. However, sensitivity (71.0%, 95% CI=53.4% to 83.9%) and NPV (55.0%, 95% CI 34.2% to 74.2%) of CRP at 40.1 mg/L were low and thus this marker should not be used as a screening tool to rule out complicated appendicitis. Although in this study, CRP >40.1 mg/L reached up to 100% specificity (95%CI 74.1% to 100.0%), this finding would clearly need external validation before it could be put into general use. The calculated specificity had a lower limit of 74.1% for the 95% CI, suggesting that there was still a reasonable chance of false positive results. Further studies are required to narrow down the range of this 95% CI. Furthermore, patients with normal CRP levels could still have simple appendicitis according to our findings. The sensitivity and specificity of CRP level in the diagnosis of all types of acute appendicitis could not be calculated in this study as we did not include the patients who were directly discharged from the ED without admission to the surgical wards.

CRP could differentiate between complicated appendicitis and simple appendicitis, but not between different subtypes of complicated appendicitis. Subgroup analysis could not be done in this study due to the small sample size. Further studies with a larger sample size are required. In our study, CRP did not help us make the decision on the type or timing of surgery. However, when CRP level was higher than 40.1 mg/L, the emergency physician and the surgeon should anticipate an increased risk of complicated appendicitis and manage the patient accordingly.

Although the age of patients in the complicated appendicitis group was apparently higher than those in the simple appendicitis group, age was not a significant factor in making the diagnosis of complicated appendicitis after multivariate logistic regression analysis. Several studies have suggested that advanced age is associated with a significant increase in complication rate up to 64.9%.^9-11^ Our investigation results actually aligned with the evidence from previous studies. A recent study in Japan suggested that the CRP level was consistent with the severity of appendicitis and could be considered as a surgical indicator marker for acute appendicitis while white cell count and neutrophil percentage could not achieve this role.^6^ In 2011, a Korean group specifically looked into the correlation of CRP with the presence of complications caused by appendicitis. They found that the CRP level was significantly higher in complicated appendicitis compared with uncomplicated appendicitis (p<0.001). In complicated appendicitis, CRP at a cut-off point of >7 mg/dL (or 70 mg/L) was not sensitive (57.6%), but it was very specific (98.3%).^7^ Similar recent work also supported the use of CRP to diagnose complicated appendicitis.^8^ The ratio of complicated to simple appendicitis, almost 3 to 1 in our study, was markedly different from usual clinical observations. This feature probably reflected the fact that we excluded patients who did not have a CRP measured in the ED, the crucial variable in this study. It is likely that there is a significant inclusion bias in this retrospective study as clinicians in our ED are more likely to request a CRP level if they suspect complicated appendicitis. According to a large American study with 3,502 patients with acute appendicitis, 33.9% were found to have complications, implying a usual ratio of 1 to 2 in favour of simple appendicitis.^9^ Therefore, the complication rate in our study was unusually high.

Although our study suggests that CRP has clinical utility in identifying complicated appendicitis, it remains inferior to abdominal computed tomography which has better sensitivity and specificity for acute appendicitis^4^ and other abdominal conditions. However, CRP is widely available and easy to perform without additional risks compared to CT. Results are
usually rapidly available and these should be particularly useful for the ED due to the short time for decision making and the lack of universal access to CT scanning. Further studies, especially prospective studies, are required to confirm our findings in other settings and in larger numbers.

**Limitations**
This retrospective study was carried out in a single centre covering all the cases within a 17-month period without blinding. Inclusion of patients could be affected by the various practices of different emergency physicians. Many emergency physicians did not take blood for CRP levels in the ED as a routine, thus producing a considerable inclusion bias. We also assumed that our emergency physicians did not miss any cases of acute appendicitis who later attended a private hospital surgeon for appendicectomy. We could not trace the medical records of patients who went for care in the private sector as this was not tracked from our systems. This study failed to evaluate the effect of time to operation on the complication rate. Although we are able to meet our overall target sample size, we were unable to include 20 patients in the simple appendicitis group as calculated. Further larger studies could improve on our results.

**Conclusions**
CRP level >40.1 mg/L may predict the presence of complicated appendicitis when acute appendicitis is clinically suspected. This finding needs to be confirmed in other settings and with a larger sample size.

**References**