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# Role of circulating fibrocytes in the diagnosis of acute appendicitis

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**Background:** **Improved diagnostic biomarkers are required for acute appendicitis. The circulating fibrocyte percentage (CFP) is increased in inflammatory states, but has not been studied in acute appendicitis. This study aimed to determine CFP in acute appendicitis and compare diagnostic accuracy with standard serological biomarkers.**

**Methods:** **A prospective cohort study was carried out between June 2015 and February 2016 at University Hospital Limerick. The CFP was determined by dual-staining peripheral venous samples for CD45 and collagen I using fluorescence-activated cell sorting, and correlated with histopathological diagnoses. The accuracy of CFP in determining histological acute appendicitis was characterized and compared with the white cell count, C-reactive protein concentration, neutrophil count, lymphocyte count and neutrophil : lymphocyte ratio.**

**Results:** **Of 95 adults recruited, 15 were healthy individuals and 80 had suspected appendicitis at presentation. Forty-six of these 80 patients had an appendicectomy, of whom 34 had histologically confirmed appendicitis. The CFP was statistically higher in patients with pathologically proven acute appendicitis than in healthy controls (median 6·1 (i.q.r. 1·6–11·6) *versus* 2·3 (0·9–3·4) per cent respectively; $P = 0·008$). The diagnostic accuracy of CFP, as determined using the area under the receiver operating characteristic (ROC) curve, was similar to that of standard biomarkers. In multinomial regression analysis, only raised CFP was retained as an independent prognostic determinant of acute appendicitis (odds ratio 1·57, 95 per cent c.i. 1·05 to 2·33; $P = 0·027$).**

**Conclusion:** **The CFP is increased in histologically confirmed acute appendicitis and is as accurate as standard serological biomarkers in terms of diagnosis.**

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## Introduction

Suspected acute appendicitis is the most common cause of acute surgical admission[1]. Despite the frequency with which the condition is encountered, accurate diagnosis of acute appendicitis continues to present a clinical challenge[2]. This is due mainly to the overlap of characteristic symptoms and signs with other intra-abdominal conditions, such as mesenteric adenitis and ovarian pathology[3–6]. This diagnostic dilemma is reflected in reported negative appendicectomy rates (NARs) that can be as high as 30 per cent in patients who undergo surgery[7,8]. These reported NARs highlight the limitations of current diagnostic tests and emphasize the need for an accurate preoperative test.

A number of studies have previously investigated the use of biomarkers including white cell count (WCC) and C-reactive protein (CRP) in the diagnosis of acute appendicitis[9–12]. Although these biomarkers are associated with low costs and are universally available, they have been criticized for lack of diagnostic accuracy in acute appendicitis[13–16]. The neutrophil : lymphocyte ratio (NLR) is another simple biomarker generated from the differential white blood cells. Studies[17,18] have demonstrated

that NLR is a useful predictor of complicated appendicitis, but has a limited diagnostic role in acute appendicitis.

Radiological investigations such as CT, MRI and ultrasound imaging have improved the diagnostic accuracy of acute appendicitis and reduced the NAR[19–21]. However, exposing patients to high levels of radiation associated with standard CT carries an increased lifetime risk of developing certain cancer subtypes[22,23]. Despite this, there is emerging evidence that low-dose CT with intestinal contrast, a standard protocol in the investigation of appendicitis, allows highly accurate diagnostic with low radiation exposure[24,25]. Ultrasound examination, on the other hand, is a less expensive and radiation-free form of imaging. However, it is operator-dependent and has a lower sensitivity for acute appendicitis than MRI and CT[26,27].

Circulating fibrocytes are haematopoietic cells derived from the bone marrow that circulate in the monocyte fraction and can differentiate into myelofibrocytes, fibroblasts and adipocytes[28,29]. They have a prominent role in both inflammatory and healing processes[30,31]. Previous work by the authors' group has demonstrated that circulating fibrocytes are increased in the mesentery in Crohn's disease, where they appear to migrate across the mesentery to access the serosal surface of the intestine[32]. They produce numerous cytokines such as tumour necrosis factor (TNF) α, interleukin (IL) 6, IL-8 and IL-10, and actively contribute to tissue remodelling by secreting fibrogenic and angiogenic growth factors such as fibroblast growth factor, endothelin 1, vascular endothelial growth factor, platelet-derived growth factor A and macrophage colony-stimulating factor[30]. The circulating fibrocyte percentage (CFP) has been shown to provide valuable diagnostic and prognostic information in inflammation-based disease processes, including idiopathic pulmonary fibrosis and bronchial asthma[33,34].

The CFP has also been speculated to play a key role in the pathogenesis of primary and secondary mesenteropathies[35,36]. To date, no studies have examined the role of CFP in the diagnosis of acute appendicitis. Thus, the initial aim of the study was to compare the CFP in patients with acute appendicitis and healthy controls. The diagnostic accuracy of CFP in diagnosing acute appendicitis compared with standard serological biomarkers was also assessed.

## Methods

A prospective cohort study was undertaken in patients aged over 16 years presenting acutely with suspected appendicitis at University Hospital Limerick, Ireland between 10 June 2015 and 14 February 2016. Blood samples were also taken from healthy controls. The diagnosis of appendicitis was confirmed by histopathological assessment after appendicectomy. Ethical approval was obtained from the Research Ethics Committee and the Quality and Patient Safety Department at University Hospital Limerick (record number 109/15), and the study was registered at ClinicalTrials.gov (NCT03988660).

A venous blood sample was collected from patients after obtaining written informed consent. Patients who had an appendicectomy were divided into three groups: patients with a histologically proven appendicitis; patients with a histologically proven normal appendix; and patients with an alternative diagnosis. Two patients had radiological features of inflammation in the right iliac fossa (1 phlegmon and 1 para-appendiceal abscess). These patients did not have surgical intervention owing to surgeon preference, and both were included in the alternative diagnosis group.

When the appendix appeared macroscopically normal at laparoscopy, it was left to the operating surgeon to decide whether an appendicectomy was required.

Data collection included date of admission, patient's sex, age, presenting symptoms, duration of symptoms, percentage of fibrocytes in circulating white cells (CFP), preoperative WCC and differentials (NLR and neutrophil, lymphocyte, monocyte, basophil and eosinophil counts), CRP level, operation performed, clinical diagnosis and postoperative histological diagnosis.

### Blood sample collection, preparation and processing

Peripheral venous samples were obtained from patients. A single 10-ml sample of heparinized venous blood was collected via peripheral arm venepuncture. Samples were collected in sodium heparin (EDTA) vacutainer tubes and transferred to the laboratory at the University of Limerick. Samples were then processed to isolate the buffy coat layer using density gradient centrifugation (Histopaque®; Sigma-Aldrich, Wicklow, Ireland). The resulting peripheral blood mononuclear cells were subsequently washed in phosphate-buffered saline (PBS) and resuspended in freezing medium (50 per cent fetal bovine serum, 40 per cent RPMI medium and 10 per cent dimethyl sulphoxide) before transfer to cryogenic vials in 1-ml aliquots. Finally, samples were cooled in a cryogenic temperature control rate container to −80°C until processing for flow cytometry.

### Sample processing for flow cytometry

After white blood cell isolation using density gradient centrifugation, $1 \times 10^6$ cells were resuspended in flow

cytometry buffer (RPMI medium supplemented with 10 per cent horse serum, 0·1 per cent sodium azide and 25 mmol/l HEPES). Cells were fixed and permeabilized using BD Cytofix/Cytoperm™ solution (BD Biosciences, Oxford, UK) and blocked before intracellular staining of collagen I with mouse antihuman collagen I antibody (product code MAB3391; Millipore, Cork, Ireland). These were then stained with Alexa Fluor® 488 goat antimouse secondary antibody (product code 115-545-146; Jackson ImmunoResearch Europe, Newmarket, UK). Cells were finally stained for cell surface antigen CD45 using PerCP® anti-human CD45 (BioLegend, London, UK) and resuspended in PBS before subsequent analysis[37–39]. All analysis was done on a BD FACSVerse™ flow cytometer (BD Biosciences) using BD FACSuite™ v1.0.5 (BD Biosciences). Circulating fibrocyte levels were displayed as a percentage of the total white blood cell population (CFP).

### Missing data

Patients presenting with suspected appendicitis who had incomplete data were not included in the final analysis.

### Statistical analysis

Data analysis was performed using IBM SPSS® for Mac OSX Version 25.0 (IBM, Armonk, New York, USA). Data are presented as mean(s.d.) or median (i.q.r.) values, as appropriate. The distribution of variables was assessed by histograms, Q–Q plots and box plots. One-way ANOVA was used to compare different independent groups. Independent *t*-test was utilized to compare normally distributed data. Kruskal–Wallis and Mann–Whitney *U* tests were used to compare the biomarkers between the different groups (appendicitis *versus* normal appendix *versus* alternative diagnosis *versus* healthy controls). $P < 0{\cdot}050$ was considered statistically significant. Pearson's correlation test was used to evaluate the relationship between the variables. Receiver operating characteristic (ROC) curves were used to characterize and compare the diagnostic accuracy of percentage fibrocytes in circulating white cells, WCC, CRP level, neutrophil count, NLR and monocyte count. Multinomial logistic regression analysis was used to assess for an independent predictor of histologically acute appendicitis.

## Results

A total of 95 participants were recruited into the study (51 female and 44 male patients). Data were incomplete for two patients presenting with suspected appendicitis (*Fig. S1*, supporting information). The study cohort was separated into the 80 patients with a clinical suspicion of acute appendicitis, and 15 healthy adult controls, who were asymptomatic with no underlying chronic medical conditions or history of appendicectomy, were recruited. Of the 80 patients presenting with suspected appendicitis, 37 (46 per cent) were men and 43 (54 per cent) were women.

**Table 1 Summary of patient demographics**

| | Men ($n = 44$) | Women ($n = 51$) | $P$† |
|---|---|---|---|
| Total (%) | 46·3 | 53·7 | 0·473‡ |
| Age (years)* | 32·5(14·2) | 36·3(13·6) | 0·190 |
| Healthy controls | 7 (47) | 8 (53) | 0·796 |
| Histologically proven appendicitis | 23 (68) | 11 (32) | 0·040 |
| Histologically proven normal appendix | 6 (50) | 6 (50) | 0·100 |
| Alternative diagnosis | 8 (24) | 26 (76) | 0·002 |
| Negative appendicectomy rate | 6 of 29 (21) | 6 of 17 (35) | 0·314 |

Values in parentheses are percentages unless indicated otherwise; *values are mean(s.d.). †$\chi^2$ test, except ‡independent *t* test.

Patient demographics are presented in *Table 1* and *Fig. S1* (supporting information). Forty-six (58 per cent) of the 80 patients underwent laparoscopic appendicectomy, with no conversions to open surgery. Of these, 34 patients had histologically proven acute appendicitis (23 men and 11 women; $P = 0{\cdot}040$). The remaining 12 patients had a normal appendix on histological assessment. The overall NAR was 26 per cent (12 of 46 patients): six of 29 in men *versus* six of 17 in women ($P = 0{\cdot}314$) (*Table 1*).

Thirty-four (43 per cent) of the 80 patients had a final alternative diagnosis, and were treated conservatively. Of these 34 patients, 16 were diagnosed with non-specific abdominal pain and four had ovarian pathology (*Table 2*). One patient was diagnosed with a phlegmon (involving the appendix, terminal ileum and related regions of mesentery) in the right iliac fossa, and another had a periappendiceal abscess. As these latter two patients were diagnosed radiologically and treated without surgery, they were included in the alternative diagnosis group.

### Association of circulating fibrocyte percentage and appendiceal inflammation

Median CFP in white cells was significantly higher in patients with acute appendicitis than in either healthy controls, patients with a normal appendix or patients with alternative diagnoses: 6·1 (i.q.r. 1·6–11·6) *versus* 2·3 (0·9–3·4), 1·7 (0·3–2·7) and 2·9 (1·4–5·8) per cent respectively ($P = 0{\cdot}003$) (*Figs 1–3*).

**Table 2 Patients with alternative diagnoses**

| | No. of patients ($n$ = 34) |
|---|---|
| Non-specific abdominal pain | 16 |
| Ovarian pathology | 4 |
| Diverticulitis | 2 |
| Terminal ileitis | 1 |
| Uterine fibroids | 1 |
| Ureteric stone | 1 |
| Constipation | 1 |
| Urinary tract infection | 1 |
| Mesenteric adenitis | 1 |
| Acute cholecystitis | 1 |
| Adhesions | 1 |
| Colonic tumour | 1 |
| Subcutaneous haematoma | 1 |
| Appendicular mass | 1 |
| Appendicular abscess | 1 |

## Association of standard serological biomarkers and appendiceal inflammation

Standard serological biomarker levels were determined for all 80 patients presenting with suspected appendicitis. Levels of each biomarker were compared between groups in multivariable analysis. Most serological concentrations of biomarkers were raised in patients presenting with suspected appendicitis (*Table 3*).

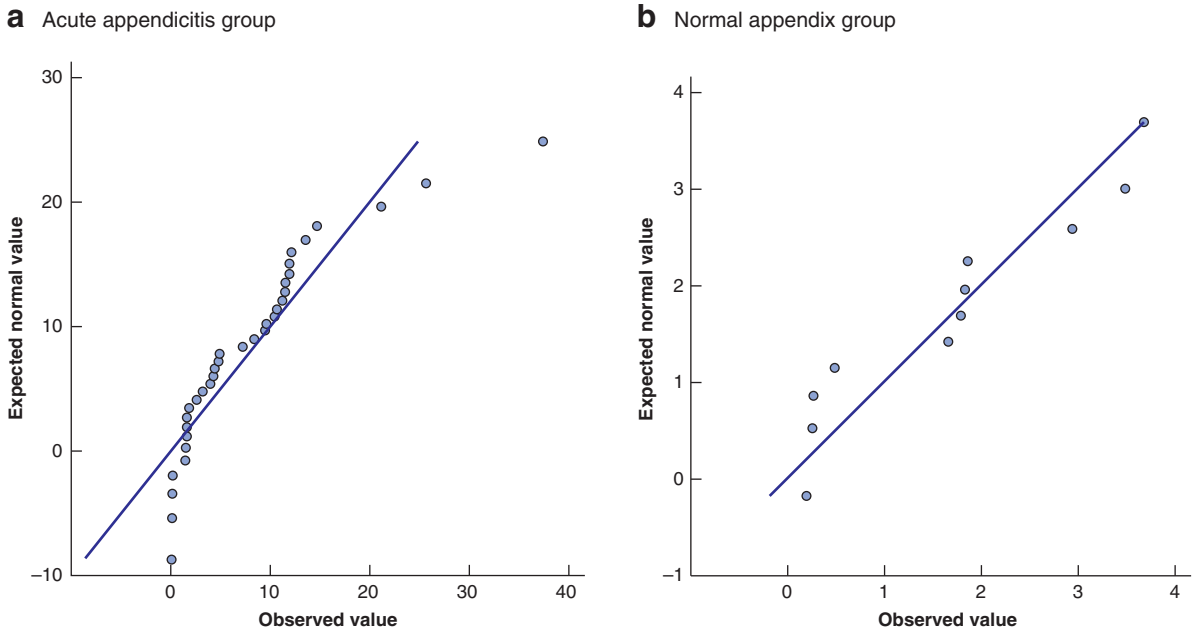
## Correlation of circulating fibrocyte levels and standard serological biomarkers

After the analysis of diagnostic accuracy analysis, a correlation analysis was performed to assess the relationships between the different biomarkers. There were significant positive linear correlations between CFP and both WCC ($r = 0{\cdot}34$, $P = 0{\cdot}002$) and neutrophils ($r = 0{\cdot}33$, $P = 0{\cdot}003$) (*Fig. S2*, supporting information). However, this linear relationship was not apparent between CFP and CRP level, NLR, monocyte count, age or sex in patients presenting with suspected appendicitis (*Fig. S3*, supporting information).

## Association of duration of symptoms and circulating fibrocyte percentage

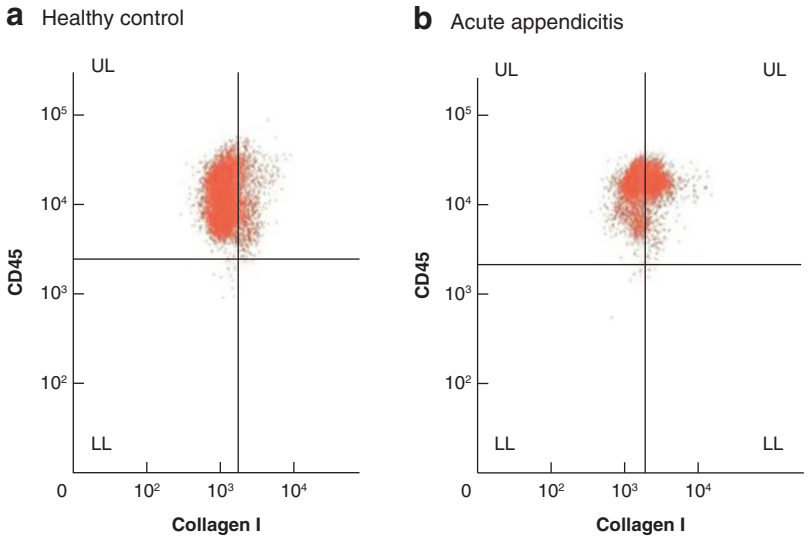
The relationship between duration of symptoms and CFP was assessed in patients with histologically confirmed acute appendicitis. Symptoms were stratified as having a duration from onset of less than 48 h and 48 h or more. Although

**Fig. 1 Q–Q plots of circulating fibrocyte percentage in patients with acute appendicitis and those with a normal appendix**



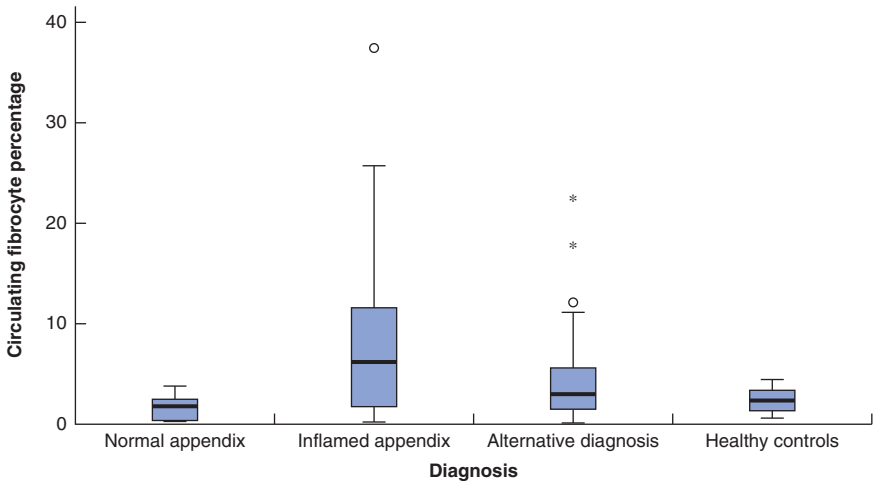

**a** Acute appendicitis group; **b** normal appendix group.

**Fig. 2 Scatter plots demonstrating differences in the percentage of circulating fibrocytes between a healthy individual and a patient with acute appendicitis**




**a** Healthy control: circulating fibrocyte percentage (CFP) 2·6 per cent. **b** Patient with acute appendicitis: CFP 8·0 per cent. UL, upper left; UR, upper right; LL, lower left; LR, lower right.

**Fig. 3 Bar chart summarizing circulating fibrocyte percentage in histologically proven normal appendix group, histologically proven appendicitis group, alternative diagnosis group and healthy controls**



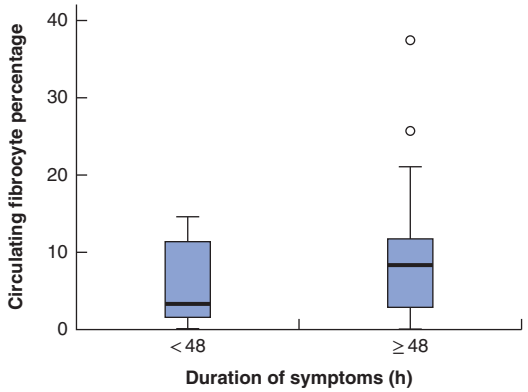

Median values, interquartile ranges and ranges (excluding outliers (○) and extreme values (*)) are denoted by horizontal bars, boxes and error bars respectively.

**Table 3 Summary of biomarker values in patients presenting with suspected appendicitis**

| | Histologically proven normal appendix | Histologically proven inflamed appendix | Alternative diagnosis | *P** |
|---|---|---|---|---|
| WCC ($10^6$/μl) | 7·0 (6·2–10·7) | 11·7 (8·2–15·5) | 8·5 (7·5–11·1) | 0·005 |
| CRP (mg/l) | 4·0 (4·0–10·0) | 16·5 (6·0–35·5) | 7·0 (4·0–33·5) | 0·016 |
| Neutrophils ($10^6$/μl) | 4·3 (3·5–8·6) | 9·3 (5·3–12·9) | 5·6 (4·7–9·3) | 0·004 |
| Lymphocytes ($10^6$/μl) | 1·8 (1·5–2·6) | 1·3 (1·0–2·0) | 1·7 (1·4–2·2) | 0·067 |
| NLR | 2·2 (1·9–4·7) | 7·1 (2·6–12·5) | 3·6 (2·0–5·9) | 0·004 |
| Monocytes ($10^6$/μl) | 0·39 (0·33–0·54) | 0·56 (0·41–0·74) | 0·44 (0·39–0·57) | 0·010 |
| Basophils ($10^6$/μl) | 0·04 (0·04–0·08) | 0·05 (0·03–0·06) | 0·04 (0·03–0·06) | 0·559 |
| Eosinophils ($10^6$/μl) | 0·16 (0·10–0·24) | 0·12 (0·06–0·20) | 0·14 (0·10–0·26) | 0·167 |

Values are median (i.q.r.). WCC, white cell count; CRP, C-reactive protein; NLR, neutrophil : lymphocyte ratio. *Kruskal–Wallis test.

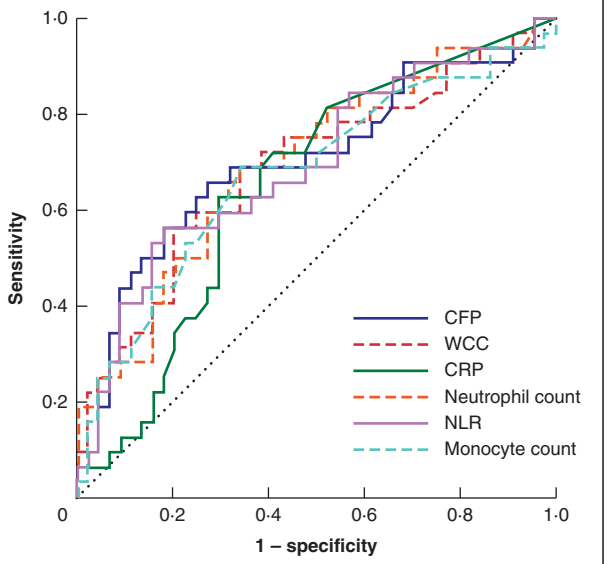
**Fig. 4 Association between duration of symptoms and circulating fibrocyte percentage in patients with acute appendicitis**




Median values, interquartile ranges and ranges (excluding outliers (○) and extreme values (*)) are denoted by horizontal bars, boxes and error bars respectively.

there was a difference between median CFP values (3·3 *versus* 8·4 per cent respectively), this was not statistically significant ($P = 0·521$) (*Fig. 4*).

**Fig. 5 Receiver operating characteristic (ROC) curves for six biomarkers**




ROC curves for circulating fibrocyte percentage (CFP), white cell count (WCC), C-reactive protein (CRP), neutrophils, neutrophil : lymphocyte ratio (NLR) and monocytes are shown.

**Table 4 Comparison of diagnostic accuracy for different biomarkers**

| | Threshold value | AUC | *P* | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|
| CFP (%) | 3·1 | 0·70 | 0·003 | 68·8 (50·0, 83·9) | 68·2 (52·4, 81·4) | 61·1 (49·0, 72·0) | 75·0 (63·3, 83·9) |
| WCC ($10^6$/μl) | 10·7 | 0·68 | 0·006 | 56·3 (37·7, 73·6) | 75·0 (59·7, 86·9) | 62·1 (47·4, 74·8) | 70·2 (60·6, 78·3) |
| CRP (mg/l) | 9·0 | 0·65 | 0·025 | 68·8 (50·0, 83·9) | 61·4 (45·5, 75·6) | 56·4 (45·5, 66·8) | 73·0 (60·6, 82·6) |
| Neutrophils ($10^6$/μl) | 7·2 | 0·69 | 0·004 | 65·6 (46·8, 81·4) | 65·9 (50·1, 79·5) | 58·3 (46·4, 69·4) | 72·5 (61·0, 81·7) |
| NLR | 4·4 | 0·69 | 0·005 | 59·4 (40·6, 76·3) | 63·6 (47·8, 77·6) | 54·3 (42·2, 65·9) | 68·3 (57·3, 77·6) |
| Monocytes ($10^6$/μl) | 0·5 | 0·67 | 0·010 | 62·5 (43·9, 78·9) | 68·2 (52·4, 81·4) | 58·8 (46·2, 70·4) | 71·4 (60·5, 80·3) |

Values in parentheses are 95 per cent confidence intervals. AUC, area under the receiver operating characteristic (ROC) curve; PPV, positive predictive value; NPV, negative predictive value; CFP, circulating fibrocyte percentage; WCC, white cell count; CRP, C-reactive protein; NLR, neutrophil : lymphocyte ratio.

**Table 5 Multinomial logistic regression analysis of different predictors in patients presenting with suspected appendicitis**

| | Odds ratio | *P* |
|---|---|---|
| **Histologically proven inflamed appendix group** | | |
| CFP | 1·57 (1·05, 2·33) | 0·027 |
| CRP | 1·01 (0·98, 1·04) | 0·410 |
| NLR | 1·10 (0·90, 1·35) | 0·323 |
| Monocytes | 21·57 (0·11, 4089·56) | 0·251 |
| Age | 0·98 (0·91, 1·05) | 0·566 |
| Male sex | 1·47 (0·26, 8·32) | 0·661 |
| Female sex* | | |
| **Alternative diagnosis group** | | |
| CFP | 1·40 (0·94, 2·08) | 0·092 |
| CRP | 1·00 (0·97, 1·03) | 0·801 |
| NLR | 1·02 (0·82, 1·26) | 0·860 |
| Monocytes | 16·31 (0·07, 3352·55) | 0·304 |
| Age | 1·04 (0·98, 1·11) | 0·166 |
| Male sex | 0·29 (0·05, 1·59) | 0·155 |
| Female sex* | | |

Values in parentheses are 95 per cent confidence intervals. Reference categories were values from the histologically proven normal appendix group. *Female sex was not included as it was a redundant parameter. CFP, circulating fibrocyte percentage; CRP, C-reactive protein; NLR, neutrophil : lymphocyte ratio.

### Diagnostic accuracy of circulating fibrocyte percentage in acute appendicitis

The accuracy of CFP in diagnosing acute appendicitis was determined and compared with that of other biomarkers using ROC curve analysis (*Fig. 5*). The performance of CFP was similar to that of the other biomarkers analysed (*Table 4*).

### Association of circulating fibrocyte percentage and risk of acute appendicitis

Given the variability of the predictors used and the lack of multicollinearity between the variables, all assumptions of the regression analysis were met. A multinomial logistic regression analysis was conducted, including all the independent predictors associated with acute appendicitis in order to characterize further the relationship between biomarker levels and the likelihood of acute appendicitis. The analysis showed that CFP was the only significant predictor of acute appendicitis (odds ratio 1·57, 95 per cent c.i. 1·05 to 2·33; $P = 0·027$) (*Table 5*).

## Discussion

This study found that the CFP of circulating white cells was increased significantly in patients with histologically proven appendicitis, compared with CFP in patients with a histologically normal appendix. Thus, increases in the proportion of fibrocytes in circulating white cells may be of diagnostic value in patients presenting with suspected appendicitis. The study also found that the diagnostic accuracy of CFP was similar to that of other commonly measured serological biomarkers used in the diagnosis of acute appendicitis such as WCC and CRP. Furthermore, when regression analysis was performed on these biomarkers, including CFP, age and sex, only CFP was retained as an independent prognostic determinant of acute appendicitis. Thus, CFP may provide useful diagnostic information in the diagnosis of acute appendicitis.

The role of CFP in acute appendiceal inflammation has not previously been assessed. This study demonstrates not only that CFP is increased in patients with acute appendicitis, but that the proportion of fibrocytes in circulating white cells may be of diagnostic value in patients presenting with suspected appendicitis. The CFP increases in response to systemic and localized inflammatory conditions[32,40–42]. Circulating fibrocytes contribute to the intestinal mesenchymal cells, which comprise a spectrum of cell types that are similar in origin, function and molecular markers. However, fibrocytes are unique because they express both haematopoietic and mesenchymal cell markers[43,44]. They can differentiate into myelofibrocytes, fibroblasts and adipocytes (as well as other cell types). Circulating fibrocytes are believed to be stimulated in response to injury of the gut mucosa[45]. Proinflammatory cytokines such as IL-6 and TNF-β are the main two components involved in recruiting leucocytes into the injured gut mucosa. In addition, the chemokine receptors CXCR4 and CCR7 are reported to be pivotal for the recruitment of fibrocytes to injured sites[46,47].

Accurate biomarkers that help with the diagnosis of acute appendicitis are lacking; WCC and CRP are the biomarkers commonly used to aid in the diagnosis of acute appendicitis[9]. These biomarkers are associated with low cost[48], but they are limited in terms of diagnostic accuracy[49,50]. Emerging biomarkers in acute appendicitis include IL-6, procalcitonin and urinary serotonin levels[11,16]. Although these have shown good diagnostic potential, they are costly[48], and thus are not currently in standard use in clinical practice.

A recent study[51] from the Right Iliac Fossa Treatment (RIFT) study group evaluated different models to identify low-risk patients who are unlikely to have acute appendicitis. This validated 15 risk prediction models in both women and men aged 16–45 years in the UK. The models evaluated were based on clinical history, examination, imaging and biochemical tests. Of the models, the Adult

Appendicitis Score achieved the highest specificity in identifying women at low risk of acute appendicitis, whereas the Appendicitis Inflammatory Response score was the optimal model in men[52,53]. Hence, specific prediction models can help to identify patients at low risk of acute appendicitis and thereby reduce the NAR. The present study shows that CFP is a predictor of acute appendicitis. It remains to be determined whether its potential utility varies between low- and high-risk groups.

At present, CT is the most sensitive and specific test for the diagnosis of acute appendicitis. However, there are concerns regarding the cost-effectiveness, accessibility and risks of radiation, and evidence that radiation from standard CT in younger patients is associated with an increased risk of soft tissue and haematological malignancy[54]. Moreover, CT is not available routinely in emergency departments. Although the CFP alone may not be as accurate as CT, it is feasible that its combination with other biomarkers could provide a scoring system with similar accuracy. This is an area for future study.

This study is limited in that it was a single-centre, cohort-based study, and did not include children as they may have a different haematological profile. The time taken to process one sample is currently 4 h, because of the different reagents and antibodies involved in the process. However, the potential of the CFP supports development of a point-of-care methodology. This could have clinical utility across a range of abdominal inflammatory conditions, not just appendicitis.

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## Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the article.