Calprotectin: The Utility Of Calprotectin In Diagnosis Of Acute Appendicitis In Pediatric Patients.

Lori Farmer

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Abstract

Objectives: Appendicitis can be a diagnostic dilemma in children. Current misdiagnosis rates in children can range as high as 57%. Accurate diagnosis of appendicitis is based upon a variety of clinical scoring systems, traditional lab biomarkers, radiological imaging studies and surgical consultations. Traditional biomarkers provide varied degrees of accuracy and predictability based on duration of symptoms, and cut-off value used. Investigators have researched several novel biomarkers, which may aid in increasing the diagnostic accuracy of traditional biomarkers with the goal of reducing CT utilization and subsequent radiation exposure risks. Calprotectin, a biomarker associated with intestinal mucosa inflammation maybe useful. What is the utility of calprotectin in the diagnosis of acute appendicitis in children with abdominal pain?

Methods: An exhaustive search was conducted through the use of Medline/Ovid, Web of Science, CINAHL, Evidence Based Medicine Review Multifile, and Google Scholar using the keywords: calprotectin, myeloid related protein(MRP 8/14), S100 proteins and appendicitis. Relevant articles were assessed for quality using GRADE. A search on the NHI clinical trials site revealed one ongoing trial related to the use of calprotectin as part of a combination biomarker panel evaluating the panels’ diagnostic accuracy in ruling out acute appendicitis in children with abdominal pain.

Results: Two studies met inclusion criteria and were included in this systematic review. A prospective, blind comparison, to a gold standard study with 503 participants that demonstrated a 3-marker panel of WBC, CRP, and calprotectin showed high sensitivity, high negative predictive value (NPV), and low negative likelihood ratios for acute appendicitis. In comparison, a combination of WBC less than 10k/ul and normal CRP had similar sensitivity and negative likelihood ratios, but reduced specificity and 14% more patients identified as false positive for acute appendicitis. A second prospective, blind comparison to a gold standard, pilot study with 176 participants demonstrated that while normal calprotectin levels showed high sensitivity and high NPV; specificity was low at negative threshold, and a normal WBC count performed better overall.

Conclusion: Calprotectin in combination with traditional inflammatory biomarkers WBC and CRP offers some benefit in the reduction of false positive test results in children with abdominal pain at sufficient low risk for appendicitis; further diagnostic radiological testing may be avoided.

Key Words: Children, appendicitis, calprotectin, MRP 8/14, S100 proteins, human

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First Advisor
Annjanette Sommers MS, PA-C

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Calprotectin: The Utility Of Calprotectin In Diagnosis Of Acute Appendicitis In Pediatric Patients.

Lori J. Farmer

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Clinical Graduate Project Coordinator: Annjanette Sommers, PA-C, MS
Biography

[Redacted for privacy]
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Table of Contents

Biography ........................................................................................................................................2
Abstract ........................................................................................................................................3
Table of Contents ..........................................................................................................................4
List of Tables ................................................................................................................................5
List of Abbreviations ....................................................................................................................5
Background ....................................................................................................................................6
Methods .........................................................................................................................................9
Results .........................................................................................................................................10
Discussion .....................................................................................................................................15
Conclusion .....................................................................................................................................20
References .....................................................................................................................................21
Tables ..........................................................................................................................................25
List of Tables

Table 1: GRADE Quality of Assessment and Recommendations.
Table 2: Summary of findings overall.
Table 3: Summary of findings related to symptom duration time.

List of Abbreviations

AUC         Area Under the Curve
CINAHL      Cumulative Index to Nursing and Allied Health
CP          Calprotectin/Myeloid-Related Protein (8/14)
CRP         C-Reactive Protein
CT          Computed Tomography
ED          Emergency Department
ELISA       Enzyme-Linked Immunosorbent Assay
GRADE       Grading of Recommendations Assessment, Development and Evaluation
LR          Likelihood Ratios
LRG         Leucine-Rich Glycoprotein
MRP 8/14    Myeloid-Related Protein (8/14)/ Calprotectin
NIH         National Institutes of Health
NPV         Negative Predictive Value
US          Ultrasound
WBC         White Blood Cell Count
Calprotectin: The Utility Of Calprotectin In Diagnosis Of Acute Appendicitis In Pediatric Patients.

BACKGROUND

Appendicitis is the most common reason for emergency abdominal surgery in children. Up to 8% of children who present urgently for evaluation of abdominal pain are diagnosed with acute appendicitis. Appendicitis occurs across all age groups, with the highest incidence presenting in the second decade of life. Although rates of appendicitis are lower in children under 5 years of age\textsuperscript{1} they are more difficult to diagnose due to their limited complex verbal skills, non-specific presentation, and often-equivocal physical exam findings. Despite intensive research and discussion, a rapid accurate diagnosis of pediatric appendicitis remains elusive.\textsuperscript{2} Symptom presentation is varied depending upon the age of the child, the duration of symptoms, and the exact position of the appendix in the abdomen.\textsuperscript{3}

Children with abdominal pain, who present with non-specific clinical exams and equivocal physical findings, can create a diagnostic quandary that challenges standard diagnostic methods. Accurate diagnosis of appendicitis is a dynamic, integrative, evaluation of symptoms combined with possible diagnostic studies to determine a test-treatment threshold. Several clinical scoring systems have been developed to assist with risk stratification for acute appendicitis in an attempt to define a specific diagnostic pathway for a child with abdominal pain. One such tool, the Pediatric Appendicitis Score (PAS), published by Samuel in 2002,\textsuperscript{4} categorizes the risk of appendicitis in children with abdominal pain using a single discriminate value on a 10-point scale.\textsuperscript{1} Observational studies have confirmed that children with a PAS
score less than or equal to 3 have a 0% to 2% risk\(^1\) of acute appendicitis. In this group, further diagnostic testing may lead to increased risk of false positive results and greater negative appendectomy rates.\(^5\) In addition, PAS scores greater than or equal to 7 are associated with a 78% to 96% risk\(^1\) for acute appendicitis and warrant surgical consultation without further diagnostic testing. The diagnostic challenge presents with PAS scores of 3 to 7, which are associated with 8% to 48% risk\(^1\) of acute appendicitis in children with abdominal pain. In these children, the options for further evaluation include, diagnostic imaging, surgical consultation, observation with serial abdominal exams and laboratory testing, or a combination of these approaches.

Over the last 20 years definitive diagnosis of acute appendicitis in children with equivocal findings has become increasingly reliant upon diagnostic imaging. Abdominal CT has been shown to have sensitivity ranges from (92% to 100%) with associated specificity ranges from (87% to 100%).\(^6\) However, concerns about a significant lifetime risk for radiation induced malignancy associated with CT exposure, especially in the pediatric population, has prompted further research into alternative diagnostic strategies.\(^4\) In an effort to decrease radiation exposure abdominal US has been recommend by the American College of Radiology, as the first choice diagnostic study in children with abdominal pain described as suspicious for acute appendicitis.\(^7\) Studies have shown abdominal US has reported high specificity ranges from (88% to 98%), but sensitivity can be suboptimal (77% to 100%).\(^6\) US is highly operator dependent and less accurate in children with obesity or a rigid abdomen.

Traditional laboratory biomarkers WBC and CRP have been extensively
evaluated as to their accuracy in detecting or excluding acute appendicitis in children. Results of these studies indicate that traditional biomarker values have sensitivity and specificity ranges that vary widely, are dependent upon symptom time duration and cut-off value used.\textsuperscript{1-6, 8-11}

Missed appendicitis is a significant concern for clinicians, who are evaluating children with non-specific findings. Currently misdiagnosis rates of acute appendicitis range from 28\% to 57\% in children 12 years old or younger.\textsuperscript{2} Missed appendicitis is also the second leading cause of malpractice judgments against emergency physicians in patients between the ages of 6 and 17 years.\textsuperscript{6} The goal of timely and accurate diagnosis for appendicitis has been, in part, a balance between perforation rates and rates of negative appendectomies.\textsuperscript{1} The challenge of diagnosis of acute appendicitis in children under the age of 5, is exemplified by perforation rates as high as 84\% and negative appendectomy rates as high as 17\%. Appendicle perforation in children is rare with symptom duration of less than 12 hours but incidence steadily increases thereafter, and is common after 72 hours\textsuperscript{1} Complications such as abscess, small bowel obstruction, prolonged hospital stays, and increased readmission rates, are frequently associated with perforated appendicitis.

Recently several novel biomarkers have been investigated as to their potential role in achieving a goal of rapid, accurate diagnosis of acute appendicitis in children, while minimizing the risks associated with ionizing radiation exposure.\textsuperscript{4,6,9} A previous study\textsuperscript{11} which included both adults and children, indicated a significant correlation between calprotectin and CRP, and calprotectin and WBC. Calprotectin is a calcium binding protein released from the cytoplasm of neutrophils in association with inflammation. It has been shown to participate in the recruitment
of leukocytes to inflamed intestinal tissue, which may make it a useful marker for acute appendicitis. Typically, in order to measure calprotectin levels, most laboratories utilize some version of an enzyme-linked immunoassays (ELISA). In a study by Mills et al investigators performed a stability study on a calprotectin ELISA. They found that precisions in immunoassay results are often highly time and processing dependent. Specifically, a processing time delay produced inflated values that increased sensitivity and decreased specificity. Recently, an investigational, rapid lateral flow assay was developed, by Venaxis Inc., which mathematically combines calprotectin with WBC and CRP to produce a single numerical value. This value may offer an improvement over individual markers in the diagnosis of acute appendicitis. This leads to the question; what is the utility of calprotectin in diagnosis of acute appendicitis in pediatric patients with abdominal pain consistent with lower risk for appendicitis?

METHODS

An exhaustive literature search for available studies, which addressed the utilization of calprotectin in children with abdominal pain when acute appendicitis is part of the differential, was conducted through the use of Medline/Ovid, Web of Science, CINAHL, Evidence Based Medicine Review Multifile, and Google Scholar. The search terms calprotectin, myeloid related protein (MRP 8/14), S100 proteins, and appendicitis were used. Studies conducted on children and in the English language were additional limitations. A further bibliography search for calprotectin or MRP 8/14 in association with appendicitis in children was performed on studies, which met inclusion criteria. After scanning abstracts for defined inclusion criteria, selected full-length texts were assessed for eligibility. Of those texts, studies were then excluded on the basis of duplication, if they were review articles and if they included a study population older
than 20 years. Further evaluation for validity and bias was conducted utilizing a critical appraisal method. Grading Recommendations, Assessment, Development, and Evaluation (GRADE)\textsuperscript{15} subjected the final articles that met all the inclusion criteria to quality assessment criteria as defined. A search on NIH\textsuperscript{16} clinical trials site revealed one ongoing study, addressing the potential diagnostic utilization of a combination biomarker panel, which incorporates calprotectin, in children, with abdominal pain suggestive of acute appendicitis. The primary study completion date was given as February 2014.

RESULTS

The initial search revealed thirty-two abstracts that were evaluated and screened for inclusion criteria. After screening, eight full-length text articles were reviewed for eligibility; of which, six were excluded for failure to meet eligibility criteria. At completion of the screening process, two prospective blind comparison to a gold standard, studies remained. Both studies were subjected to further systematic review.\textsuperscript{6,10} (See Table 1.)

A Novel Biomarker Panel to Rule Out Appendicitis in Pediatric Patients With Abdominal Pain.

This prospective blind comparison to a gold standard study\textsuperscript{6} investigated a new biomarker panel with potential sufficient sensitivity and negative predictive value to allow for successful identification of children with abdominal pain, at low risk for appendicitis, in order to avoid unnecessary imaging. The study enrolled 569 pediatric patients with abdominal pain, who presented at 12 different, emergency departments located in children’s hospitals, tertiary care centers, and community hospitals. Sixty-Six patients were excluded for ineligibility, lack of adequate test samples, or invalid test results. Of the remaining 503 participants,
351 came from large academic children’s hospitals or tertiary care centers. Eligibility criteria included children, age 2 to 20 years, with right lower quadrant or generalized abdominal pain associated with additional signs and symptoms suspicious for or consistent with acute appendicitis and symptom duration of less than 72 hours.\textsuperscript{6}

The primary study intentions were to analyze the diagnostic accuracy of individual plasma biomarkers and combinations of such markers to rule out acute appendicitis in pediatric patients with abdominal pain and whose differential diagnosis included appendicitis. The gold standard reference measurement of primary outcomes was the presence or absence of acute appendicitis, defined by a surgical pathology report for those who had an appendectomy and a discharge diagnosis for those without an appendectomy. Follow-up on patients discharged with a diagnosis other than appendicitis, consisted of a review of the hospital records of all enrolled patients for return visits within 72 hours of discharge; any discharged patient who returned to the same institution with ongoing symptoms would be captured. The secondary outcomes and end points were the utilization rate of CT scanning and potential reduction of unnecessary CT scans for those with negative biomarker results should the biomarkers provide adequate diagnosis for absence of acute appendicitis.\textsuperscript{6}

After an in-depth post hoc analysis of several individual plasma biomarkers and combinations of such markers, investigators discovered that the combination of WBC, CRP, and MRP 8/14 (also known as calprotectin) provided an optimum panel. This biomarker panel used three individual markers combined with a mathematical algorithm which were converted into single value with an appropriate cut-off that provided a diagnostic tool with a high negative predictive value (NPV). The cut-off was selected for clinical utility maximizing the number of true negative test results, while
minimizing the number of false negative results. Serum samples were obtained on all participants based solely on presenting symptoms, thereby avoiding any bias that might be introduced by a preceding diagnostic evaluation. The samples were initially centrifuged, frozen, and sent to a research laboratory where they were later thawed and tested using a new MRP 8/14 lateral flow assay developed by Venaxis, Inc.\textsuperscript{6}

The biomarker panel identified 160 patients out of 503 as negative, of those 155 were true negatives and 5 were false negatives. Biomarker panel sensitivities, specificities, NPV and likelihood ratios are listed in Table 2. Study investigators also compared the algorithm to a combination of normal WBC (<10 k/ul) and, normal CRP (<0.8 mg/dl). Used as a negative predictor when both WBC and CRP were normal, sensitivities and NPV were comparable to the biomarker algorithm, but specificity was diminished and there were 34 fewer patients identified as true negatives with one fewer false negative.\textsuperscript{6} (See Table 2.)

Further examination of the outcomes, revealed a significant increase in sensitivity and NPV with duration of symptoms lasting between 24 to 48 hours. However, specificity values remained unchanged (see Table 3). Of note, is the fact that all five of the patients with false negative biomarker panel results had a symptom duration of less than 24 hours, including two with symptoms for less than 12 hours.\textsuperscript{6}

The secondary outcome measure of the potential impact of the biomarker panel on reduction of CT utilization, indicated that 185 out of 503 patients in the cohort received a CT scan. Of those, 60 patients had a negative biomarker panel, which represented 32.4\% of all CT scans preformed. If those patients had been followed clinically rather than referred for immediate CT imaging, there could have been a potential reduction of 1/3 in CT utilization at initial presentation. The
authors did, however, note that this estimate may be generous, as some patients with negative biomarker results may still be referred for CT imaging if there is high clinical suspicion, or other pathology in addition to acute appendicitis being considered.⁴

The authors primary concern was the lack of formal follow-up for those patients discharged from the Emergency Department (ED) with a diagnosis other than acute appendicitis. They acknowledged that due to the small number of patients with acute appendicitis and false-negative biomarker results, a single missed false-negative patient could have a significant effect on the results of the study.⁶

**Novel Serum And Urine Markers For Pediatric Appendicitis**

This prospective, blind comparison to a gold standard pilot study,⁹ examined two novel biomarkers, calprotectin and leucine-rich glycoprotein-1 (LRG), for the purpose of determining the diagnostic relationship between serum and urine levels of these biomarkers and appendicitis in children with abdominal pain. Study researchers also investigated the optimal thresholds of each marker that could potentially be used to diagnose or exclude acute appendicitis.⁹

The study enrolled 176 patients with abdominal pain who presented to an urban, tertiary care, pediatric emergency department from July 2009 to April 2010. All study participants had a standard WBC drawn in addition to investigational biomarker samples. Plasma samples for calprotectin analysis were successfully obtained on 153 patients. Urine samples for calprotectin were obtained on 137 patients. Eligibility criteria included children with abdominal pain less then 96 hours’ duration, aged 3 to 18 years and with possible appendicitis. Investigators defined “possible appendicitis” as the treating physician choosing to obtain blood tests, radiologic studies, CT and/or US, or surgical consultation for the
The primary study intentions were to evaluate the association of calprotectin and LRG with acute appendicitis, and to identify potential thresholds, which could be used to diagnose or exclude appendicitis. The primary gold standard outcome measurement was the presence or absence of appendicitis defined by a histopathology report of a positive surgical appendectomy. For patients who did not have surgery, this outcome was determined by a follow-up telephone call 14 days to 21 days after the index ED visit. Investigators completed follow-up on 99% patients who did not undergo surgery.

The 153 plasma samples obtained on enrolled study participants were initially centrifuged, during weekdays from 09:00 to 16:00 and were immediately frozen. During evenings and weekends, samples were initially centrifuged, then stored at 4 degrees Celsius, until the next business day when they were frozen. Frozen samples were later sent to a research laboratory where they were thawed and analyzed utilizing ELISA according to the manufacturer’s recommended procedures.

ELISA results indicated median serum levels of calprotectin exhibited statistically significant elevations in association with acute appendicitis. When stratified according to severity of disease, plasma levels of calprotectin were highest in children with perforated appendicitis compared to non-perforated appendicitis. Following a similar pattern, levels remained higher in non-perforated appendicitis compared to non-appendicitis. However, urine calprotectin levels showed no statistical difference among the groups.

Determining area under the curve (AUC), assessed accuracy for each biomarker. The AUC for serum calprotectin was (0.68, 95% CI 0.59 to 0.79). In comparison the
AUC for the WBC count was notably higher (0.82 95% CI 0.75 to 0.90). Threshold levels for calprotectin were determined which provided 100% sensitivity, although the specificity remained low (see Table 2). Study investigators also completed an exploratory analysis of a combination of WBC and plasma calprotectin which led to improved specificity; however, sensitivity remained unchanged at 100%.9

Lab personnel, who were blinded to the diagnosis of enrolled patients, detected calprotectin levels via a commercially available ELISA kit. The assay required a 4-hour processing time to be completed in a research laboratory with particular expertise in biomarker discovery. Investigators also expressed concern that the small size of the study population limited the strength of their preliminary, exploratory results that suggested a potential benefit of combining calprotectin with a standard WBC. To be clinically useful, results would need to be validated by larger studies.

DISCUSSION

Children, who present with unequivocal clinical exams for the presence or absence of acute appendicitis often, provide a clear diagnostic path for the clinician to follow. Adversely, children, who present with equivocal findings, can be challenging to diagnose and can have potentially high-risk outcomes associated with misdiagnosis. Utilization of diagnostic CT imaging has provided accurate, highly sensitive and specific diagnostic results but is associated with significant increased malignancy risks.6,4,17 Ultrasound has optimal accuracy if visualization of appendicitis is achieved, but is less efficient at reassuring absence with lack of visualization. Traditional inflammatory biomarkers WBC and CRP are a much lower risk diagnostic approach. However, in children, they have provided varied degrees of sensitivity and specificity depending on cut-off values used and duration of symptoms present. Investigators have been exploring
other possible biomarkers such as calprotectin, which may improve the accuracy of traditional biomarkers ability to differentiate which children need to undergo additional potentially higher risk diagnostic evaluations.

The results from the two studies reviewed,\textsuperscript{6,9} indicated that calprotectin exhibited high sensitivity, high negative predictive value and low negative likelihood ratios both individually and in combination with traditional biomarkers WBC and CRP. (See Table 2.) A combination of normal WBC (<10 k/ul) and CRP results, also exhibited statistically similar sensitivity, negative predicative value, and low negative likelihood ratios. Although, the biomarker algorithm did increase specificity over the combination of normal WBC (<10 k/ul) and CRP and allowed for a 14% reduction in false positive patients with an increase of one more false negative patient. Significantly, investigators noted that all false negative patients had symptoms present for less than 24 hours.\textsuperscript{6} In addition, the study by Kharbanda et al\textsuperscript{9} noted that a normal WBC (<8.85 k/ul), provided both a 100% sensitivity and a higher specificity when compared to a normal calprotectin value. Kharbanda et al\textsuperscript{9} also performed an exploratory analysis of combined normal calprotectin and WBC. Results of combination again showed good sensitivity with an increased specificity of 52% (no confidence intervals provided). Both of these studies,\textsuperscript{6,9} demonstrated an increase in specificity with the addition of calprotectin in the diagnostic approach. This translated into a lower rate of false positive results potentially leading to fewer children undergoing unnecessary diagnostic imagery.

Duration of symptom time appears to be a significant factor, in regards to the estimated sensitivity and negative predictive values of inflammatory biomarkers. Distinctly, in respect to the combination biomarker panel (calprotectin, WBW, and CRP) optimal sensitivity was achieved in patients with symptom duration from 24 hour to 48
hours in length. Nonetheless, specificity values remained unchanged and confidence intervals narrowed with symptom duration greater than 24 hours. See Table 3. Huckins et al. did not perform an additional sub-group analysis of the biomarker algorithm panel to the combination of normal WBC (<10 k/ul) and CRP based on time of symptom duration. Kharbanda et al. did not address variability in accuracy of sensitivity of calprotectin and WBC count related to symptom duration. While not addressed in these studies, further investigation into the effect of symptom duration on sensitivity, negative likelihood ratios, and NPV of optimally determined normal, combined WBC and CRP values, as compared to the three marker panel, may provide an additional insight into the potential benefit of a calprotectin incorporated panel over traditional biomarker combinations.

The type of biomarker assay method used may be another critical factor to consider in determining the diagnostic accuracy and efficiency of calprotectin. The Huckins et al. study, utilized a new investigational lateral flow assay panel, which mathematically combined the three identified biomarker values (WBC, CRP and calprotectin) into a single numerical value. The panel was designed to provide rapid, reproducible results in urgent or emergent health care settings with the goal of effectively ruling out the presence of acute appendicitis in children with abdominal pain, who are at lower risk for acute appendicitis; reducing the need for further radiological diagnostic studies. The Kharbanda et al. study, utilized a commercially available ELISA assay which measured individual calprotectin levels and provided quantified results. The assay took 4.5 hours to complete, in a research laboratory, which can be an obstacle for the clinical goal of rapid exclusion of appendicitis in children with abdominal pain and equivocal clinical findings. Both studies utilized serum samples for the assay that were
obtained at the time of enrollment in the ED, centrifuged locally, frozen and assayed later in research laboratories. An important concern is that the samples tested were not fresh on-site samples as would be typically be done in clinical practice. For instance, in a recent study by Mills et al\textsuperscript{13} which utilized an investigational calprotectin ELISA, investigators found that despite careful adherence to manufactures recommended processing methods, measured values can increase by 13\% to 43\% due to shipping effect and delay in analysis.\textsuperscript{13} If these values were not addressed and adjusted for, falsely elevated sensitivity and falsely decreased specificity values occurred.

Both studies indicated that calprotectin levels are elevated in children with acute appendicitis and may have a role improving the reliability and accuracy of traditional inflammatory biomarkers as negative predictors in children.\textsuperscript{6,9} However, both studies also had several limitations: First, both studies utilized cohorts from convenience sampling methods which can be problematic; there may be a difference between people who choose to participate and those who do not. Second, both of the study settings were disproportionately located in urban, specialty hospitals where the incidence of children with appendicitis and availability of diagnostic resources may be different from community hospitals.\textsuperscript{6,9} Third as previously addressed above, both studies used frozen samples assayed at a later date, which may affect accuracy of results.

Moreover, the Huckins et al\textsuperscript{6} study, was an industry funded, futility analysis looking at the accuracy of an investigational rapid biomarker panel which has been patented and is currently being marketed to investors explicitly for the purpose of rapid accurate exclusion of acute appendicitis in children who present with abdominal pain and have lower risk profiles for appendicitis. Another significant limitation to this study\textsuperscript{6} is the lack of formal follow-up on patients discharged. Because of the
small number of patients with acute appendicitis, a single missed false negative patient would have significant effects on study outcomes. The Kharbanda et al study, was a single-center, pilot study with a small sample size. Small population samples can have the potential to lead to lack of precision in results. The study also primarily evaluated the diagnostic accuracy and utility of calprotectin and WBC as individual biomarkers and only addressed the potential benefit of combined WBC and calprotectin as part of a preliminary exploratory analysis with limited data published. Therefore, after a detailed assessment of both studies strengths and limitations, an overall combined quality of evidence of the articles reviewed is low to very low, as based upon the GRADE criteria. A weak recommendation for the utility of calprotectin in diagnosing acute appendicitis can be given based upon the low quality evidence supporting the benefit of combined calprotectin, WBC and CRP over the use of standard WBC and CRP.

CONCLUSION

In children, who have abdominal pain and an equivocal clinical exam for acute appendicitis, biomarker panel results (calprotectin, WBC and CRP) offered some benefit over WBC and CRP values in reducing false positive results for acute appendicitis; potentially decreasing the need for further radiological testing. Notably, in the same population of children, biomarker panel results (calprotectin, WBC and CRP) demonstrated only moderate accuracy in identifying true negative results; thereby, leaving a significant population of children, who may likely require further radiological testing. Further research, which is currently ongoing is likely to have an important influence on the confidence of the recommendation and may change the recommendation.


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<tr>
<th>Table 1 GRADE Quality Assessment And Recommendation</th>
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**Quality Assessment**

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<th>Downgrade Criteria</th>
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<tr>
<td>Design Limitations</td>
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<td>Indirectness</td>
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<td>Imprecision</td>
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<td>Inconsistency</td>
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<td>Publication bias likely</td>
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**Huckins et al.**

A novel biomarker panel to rule out acute appendicitis in pediatric patients with abdominal pain

- Prospective Blind comparison to a gold standard: Very serious limitations
- No serious indirectness
- No Serious imprecision
- No serious inconsistency
- Publication bias likely

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<thead>
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<td>Huckins et al.</td>
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**Kharbanda et al.**

Novel Serum and Urine Markers for Pediatric Appendicitis

- Prospective Blind comparison to a gold standard
- Serious limitations
- No serious indirectness
- Serious limitation
- No serious inconsistencies
- No bias likely

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*a Convenience non consecutive sampling

*b Industry funded study with 5 of the 6 study investigators indicate positive conflict of interest statements

*c Convenience non consecutive sampling

*d Small sample size, Single center pilot study
TABLE 2 Summaries Of Overall Findings

<table>
<thead>
<tr>
<th>Study</th>
<th>Biomarker Diagnostic test</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td>Huckins et al</td>
<td>Biomarker panel: WBC, CRP, MRP</td>
<td>Sensitivity: 96.5% (95% CI, 92%-99%)</td>
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<td></td>
<td>8/14/calprotectin</td>
<td>Specificity: 43.2% (95% CI, 38%-48%)</td>
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<td>WBC (&lt;10 k/ul) + CRP (&lt;0.8 mg/dl)</td>
<td>Negative Predictive Value: 96.9% (95% CI, 93%-99%)</td>
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<td>LR-: 0.081</td>
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<td>LR+: 1.70</td>
</tr>
<tr>
<td>Kharbanda et al</td>
<td>Plasma calprotectin (&lt;159 ng/ml)</td>
<td>Sensitivity: 100% (95% CI, 91%-100%)</td>
</tr>
<tr>
<td></td>
<td>WBC (&lt;8.85% k/ul)</td>
<td>Specificity: 27% (95% CI, 19%-37%)</td>
</tr>
<tr>
<td></td>
<td>Exploratory analysis: WBC + calprotectin</td>
<td>Negative Predictive Value: 100% (95% CI, 81%-100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LR-: 0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LR+: 1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LR+: 1.72</td>
</tr>
</tbody>
</table>

**Sensitivity:** True Positive Results / (True Positive Results + False Negative Results) = True Positive Rate, the percentage of positive test results in patients who have the disease. Negative results with high sensitivities rules diagnosis out

**Specificity:** True Negative Results/ (True Negative Results + False Positive Results) = True Negative Rate, the percentage of negative test results in patients who do not have the disease. Positive results with high specificities rules diagnosis in

TABLE 3 Summaries Of Duration Of Symptoms Biomarker Panel Findings

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration of symptoms</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huckins et al</td>
<td>&lt;24 hours</td>
<td>Sensitivity: 94.4% (95% CI 88%-98%)</td>
</tr>
<tr>
<td></td>
<td>&gt;24 hours</td>
<td>100% (95% CI 94%-100%)</td>
</tr>
</tbody>
</table>

Sensitivity: True Positive Results / (True Positive Results + False Negative Results) = True Positive Rate, the percentage of positive test results in patients who have the disease. Negative results with high sensitivities rules diagnosis out

Specificity: True Negative Results/ (True Negative Results +False Positive Results) = True Negative Rate, the percentage of negative test results in patients who do not have the disease. Positive results with high specificities rules diagnosis in