ISSN: 3065-4483
Research Article
Science Set Journal of Pediatrics

The Significance of Combined Detection of Ultrasonography, Pediatric Appendicitis Score and C-Reactive Protein in the Diagnosis and Pathological Type of Acute Appendicitis in Children

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Submitted: 11 April 2024 **Accepted:** 15 April 2024 **Published:** 22 April 2024

https://doi.org/10.63620/MKSSJP.2024.1012

Citation: *Hai Hu, Minghui Lin, Wei Li, Jiabo Chen, Congjun Wang, et al. (2024) The Significance of Combined Detection of Ultrasonography, Pediatric Appendicitis Score and C-Reactive Protein in the Diagnosis and Pathological Type of Acute Appendicitis in Children. Sci Set J of Pediatrics 2(2), 01-08.*

Abstract

Background: *Timely diagnosis of acute appendicitis, a common acute abdominal condition, is crucial. This study assesses the diagnostic value of ultrasonography, Pediatric Appendicitis Score (PAS), and C-reactive protein (CRP) in children.*

Methods: *A retrospective analysis of 268 children with acute abdominal pain between January 2017 and 2020 categorized them into acute and non-acute appendicitis groups based on pathology. The acute group was further classified into simple, suppurative, and gangrenous types.*

Results: *Among 150 patients with acute appendicitis, PAS and CRP were significantly higher than in the non-acute group. The AUCs for ultrasonography, PAS, and CRP were 0.897, 0.871, and 0.777, respectively. The combination of ultrasonography with PAS and CRP increased sensitivity and negative predictive value but reduced specificity and positive predictive value.*

In complicated appendicitis, CRP levels and the AUCs for CRP and ultrasonography were notably higher. The sensitivity for diagnosing complicated appendicitis with ultrasonography and CRP combined was 98.21%, superior to either alone. Ultrasonography sensitivities for various pathological types were 78.95%, 81.97%, and 92.16%, with diagnoses aligning with pathology (Kappa=0.888).

Conclusion: *Combining ultrasonography, PAS, and CRP enhances the diagnosis of acute appendicitis in children and helps identify pathological subtypes, aiding clinical decision-making.*

Keywords: Acute Appendicitis, Children, Ultrasonography, Pediatric Appendicitis Score, C-Reactive Protein, Histopathology

Introduction

Acute appendicitis was one of the most common acute abdominal diseases in pediatric surgery, 1–8% of which diagnosed with acute appendicitis [1]. The onset peaks age of acute appendicitis in children is 10 years old [2]. Acute appendicitis is classified into two forms: simple and complicated. The simple form is the most prevalent and presents with less severity, marked by appendiceal inflammation without associated complications such as necrosis, perforation, or purulent fluid accumulation in the abdomen.

Complicated appendicitis encompasses scenarios where the appendix is inflamed and purulent material forms, either within the organ (acute suppurative appendicitis) or in the adjacent tissues. It also includes acute gangrenous appendicitis, where the appendix tissue dies due to compromised blood flow from inflammation, and acute perforated appendicitis, characterized by a rupture that allows purulent contents and intestinal matter to enter the peritoneal cavity [3]. For acute simple appendicitis (except with calculus), conservative treatment with antibiotics is considerable, while acute complicated appendicitis requires surgical treatment [4].

Perforation is the most concerning complication of acute appendicitis, which may lead to abscesses, peritonitis, bowel obstruction, fertility issues, and sepsis [5]. Research showed the rate of missed diagnosis for children under 3 years old was fairly high (70%-100%), and 19%-57% for preschool children, which declined to 12-28% for school-age children [6]. Consequently, the early diagnosis and assessment of pathological types of acute appendicitis are of great significance for making decisions for treatment.

PAS is a scoring system developed in a prospective cohort study of 1170 patients by Madan Samuel in 2002 and contains 8 variables that are statistically significant for acute appendicitis [7]. Derived from visceral adipose tissue during acute inflammation, the expression of CRP related to severity of inflammation [8]. Studies showed that CRP ≥ 10mg/L could be a strong predictor of acute appendicitis in children under 6 years of age [9]. Imaging examinations also play prominent roles in the diagnosis of acute appendicitis. Characterized by simplicity, economy, free of ionization radiation, high sensitivity and specificity for the diagnosis of acute appendicitis, and value for distinguish different pathological types, ultrasonography was wildly used in children with suspected appendicitis in spite of many distractions like obesity, intestinal gas [10, 11].

Guidelines for the management and treatment of acute appendicitis discussed and developed by the World Society of Emergency Surgery (WSES) in 2015, updated again in July 2019, suggested that combining ultrasonography with clinical scoring may significantly improve diagnostic sensitivity and specificity [4, 12]. Nevertheless, studies that integrate clinical scores, inflammatory markers, and imaging for diagnosing acute appendicitis in pediatric patients are scarce.

The combination of ultrasonography with the PAS and CRP may offer a promising approach for early detection of acute appendicitis in children, as all these elements have been significantly correlated with the condition and may aid in determining its severity. In this investigation, the clinical data from 268 pediatric patients suspected of having acute appendicitis were retrospectively analyzed to evaluate the effectiveness of ultrasonography, PAS, and CRP in the early diagnosis of the disease.

Information and Methods

Subjects

A total of 268 patients suffering acute abdominal pain, visited Department of Pediatric Surgery of our central between 2017 and 2020, were retrospectively analyzed. The patient cohort, comprising 154 males and 114 females, spanned 2 to 14 years of age with a mean age of 6.88 years (±1.98 standard deviation). Participants were classified into two groups based on their final diagnosis: Group AA for acute appendicitis and Group NAA for non-acute appendicitis.

Group AA was further subdivided into Group A (38 cases of simple appendicitis), Group B (61 cases of suppurative appendicitis), and Group C (51 cases of gangrenous appendicitis). This study, involving human subjects, was reviewed and approved by the ethics committee of The First Affiliated Hospital of Guangxi Medical University. Exclusion criteria included: (1) individuals over 16 years of age; (2) a diagnosis of chronic appendicitis; (3) abdominal pain due to abdominal trauma or surgery; (4) incomplete clinical data for children.

Methods

Upon obtaining consent from the patients or their guardians, a complete blood count, CRP assessment, and ultrasonography were conducted promptly for patients admitted to our facility. The complete blood count was determined using a Beckman-Coulter LH750 Automatic Blood Cell Counter, while the CRP levels were quantified via a rate turbidimetric assay on a Beckman ARRAY360 specific protein analyzer. Both tests were carried out by the Laboratory Department of our institution.

All ultrasound examination reports were provided by the Department of Ultrasound at our facility. The PHILIPS EPIQ5 color Doppler ultrasound system was utilized for the assessment of suspected acute appendicitis, employing a low-frequency probe with a 5.0 MHz setting and a high-frequency probe ranging from 10.0 to 14.0 MHz the abdomen was scanned in multiple sections from the inferior hepatic margin to the pelvis using ultrasound probes, with particular attention to tender areas while patients were in the supine position. In cases of older children with thicker abdominal walls, a low-frequency probe was initially used to identify potential areas of concern, followed by a high-frequency probe for detailed examination and confirmation.

The examination assessed various aspects, including the appendix's location, shape, maximum diameter, wall structure and continuity, lumen echo, adjacent tissues, periapendicular effusion, organ adhesions, and any surrounding masses. The highest quality images were selected for documentation, and the ultrasound features were subsequently recorded. The ultrasound characteristics of a normal appendix and the different types of appendicitis are detailed as follows (refer to Table 1 and Figure 1).

Table 1: Final diagnosis for the 268 patients

Final Diagnosis	No.
Simple appendicitis	38
suppurative appendicitis	61
Gangrenous appendicitis	51
Lymphadenitis	28
Omental torsion	17
Diverticulitis, enteritis	15
Gynecologic conditions	21
Urinary infection	25
Other surgical condition	12
Total	268

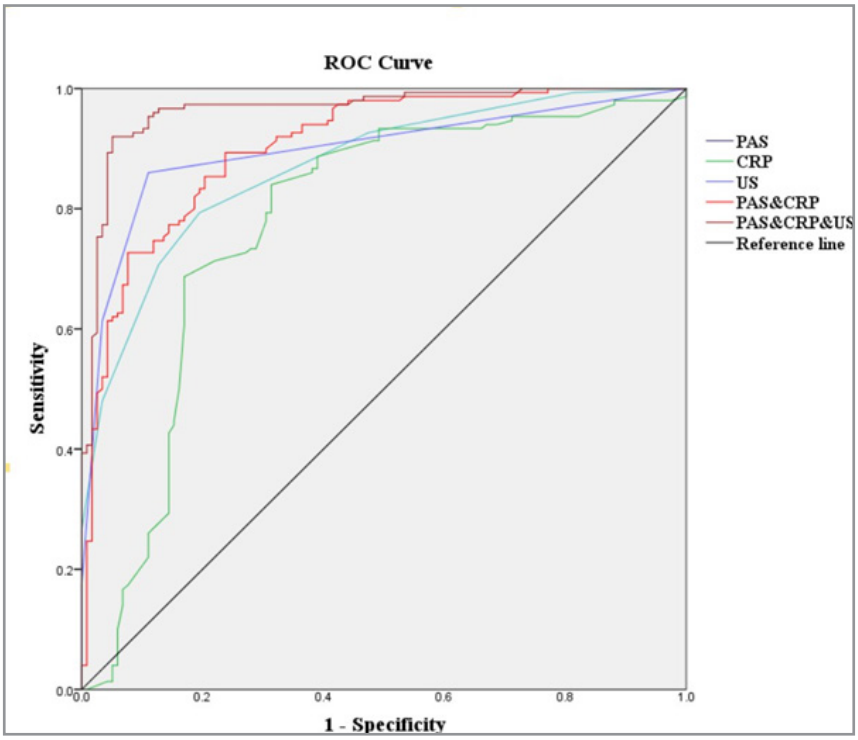

Figure 1: ROC curve of Pediatric appendicitis score and CRP for diagnosing acute appendicitis

The PAS assists in diagnosing appendicitis by assigning scores to a child's symptoms, physical examination findings, and biochemical test results. The assessment items include migration of pain (assigned 1 point), anorexia (assigned 1 point), nausea or vomiting (assigned 1 point), tenderness in the right lower quadarant of the abdomen (assigned 2 points), pain with cough/hopping/percussion (assigned 2 points), elevated temperature (assigned 1 point), leukocytosis (assigned 1 point), differential white blood cell count with a left shift (assigned 1 point) and the higher the PAS of patients, the higher the risk of acute appendicitis [13]. In this study, PAS appraised independently by two pediatric surgeons, both with substantial clinical experience. In instances where disagreements occurred, a third pediatric surgeon was enlisted to participate in the evaluation process until a unanimous consensus was achieved.

Statistical Methods

The statistical analysis was performed using SPSS 24.0 software, with a P-value of less than 0.05 set as the threshold for statistical significance. Continuous variables with a normal distribution were presented as the mean ± standard deviation (x ± SD), while those not normally distributed were depicted as the median (interquartile range). Categorical variables were expressed in terms of percentages (%).

The Shapiro-Wilk test was employed to assess the normality of the data distribution, confirming normality with P-values less than 0.001. For comparing continuous variables between groups, the independent samples t-test was utilized, and the chi-squared test was applied for categorical variables. The diagnostic accuracy of the examinations for different types of appendicitis in children was assessed using the receiver operating characteristic (ROC) curve. The consistency between ultrasonographic diagnoses and pathological findings in children with various pathological types of appendicitis was evaluated using the Kappa test.

Results

General Data

The definitive diagnoses for 268 patients are presented in Table 2. Of these, 150 patients diagnosed with acute appendicitis were classified into Group AA, and the remaining patients into Group NAA. A comparative analysis of the general data between the two groups is detailed in Table 3. No significant differences were observed in terms of age, gender, height, and weight (P>0.05).

Table 2: Comparison of general date, PAS and CRP between the group NAP and group AP

Genral Data	NAA(N=150)	AA(N=118)	*t* or x^2	p value
Gender(male,%)	58.00	56.78	0.400	0.841
Age(Mean ± SD, year)	6.97 ± 2.04	6.81 ± 1.94	0.0.626	0.532
Weight(Mean ± SD, kg)	27.53 ± 7.69	26.87 ± 7.47	0.713	0.476
Height(Mean ± SD, cm)	118.90 ± 13.41	118.11 ± 12.99	0.610	0.629
PAS (Mean ± SD)	4.64 ± 1.33	7.22 ± 1.69	9.269	< 0.001*
CRP(Mean ± SD, mg/L)	26.24 ± 11.50	35.71 ± 9.46	3.073	< 0.001*

CRP, C-reactive protein; PAS, Pediatric appendicitis score;
NAA, group non-acute appendicitis; AA, group acute appendicitis
* Means P-value < 0.05

Table 3: Value of modified PAS, CRP and ultrasonography in the diagnosis of acute appendicitis

Diagnostic methods	Sensitivity (%)	Specificity (%)	Positive predictive value(%)	Negative predictive value(%)	Positive likelihood ratio	Negative likelihood ratio	Youden index
PAS (≥ 6) a	82.67 (124/150)	89.83 (106/118)	91.18 (124/136)	80.30 (106/132)	8.129	0.193	0.725
CRP (≥ 27.06mg/L) a	86.00 (129/150)	71.19 (84/118)	79.14 (129/163)	80.00(84/105)	0.985	0.197	0.572
Ultrasonography	88.39 (137/150)	88.50 (100/118)	91.33 (137/155)	84.75 (100/113)	7.683	0.131	0.769
a means the cutoff value of PAS and CRP in judging acute appendicitis was 5.5 and 27.06 mg/L, respectively							

Analysis of Diagnostic Methods

PAS, CRP, and Ultrasonography in Acute Appendicitis

In the comparison between Group AA and Group NAA, both the PAS and CRP levels in Group AA were significantly elevated compared to those in Group NAA (P<0.05; refer to Table 3). To further assess their diagnostic efficacy, the ROC curves for ultrasonography, PAS, and CRP were plotted for the diagnosis of acute appendicitis (refer to Figure 2). The respective areas under the ROC curves (AUCs), with a 95% confidence interval, were 0.897 (0.857-0.937) for ultrasonography, 0.871 (0.829-0.912) for PAS, and 0.777 (0.716-0.838) for CRP (P< 0.05). The optimal cutoff points for PAS and CRP, as determined by the Youden index, were identified as 6 and 27.06 mg/L, respectively. An analysis of the diagnostic performance of these methods indicated that ultrasonography had the highest diagnostic value, evidenced by the largest AUC and the most substantial Youden index (refer to Table 4).

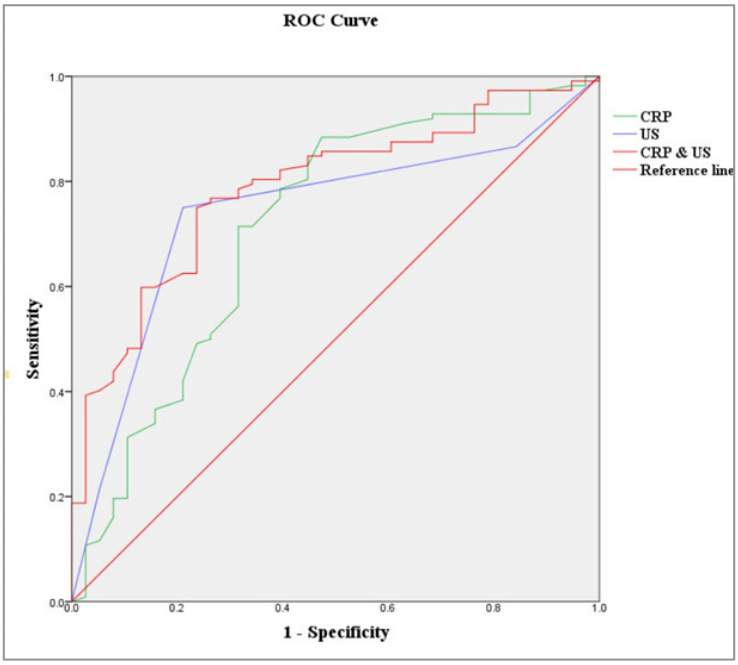

Figure 2: ROC curve of PAS and CRP for diagnosing acute complicated appendicitis

Table 4: Value of combination of ultrasonography, PAS and CRP in the diagnosis of acute appendicitis

Diagnostic methods	Sensitivity(%)	Specificity (%)	Positive predictive value(%)	Negative predictive value(%)	Youden index
Ultrasonography alone	88.39 (137/150)	88.50 (100/118)	91.33 (137/155)	84.75 (100/113)	0.769
Combination of ultrasonography, PAS and CRP	99.33 (149/150)	54.24 (64/118)	73.40 (149/203)	98.46(64/65)	0.536
χ - value	10.789	29.804	12.289	5.656	
P- value	0.001*	< 0.001*	< 0.001*	0.017*	
* Means P-value < 0.05					

Combined with Three Methods to Diagnose Appendicitis

The combined use of ultrasonography, PAS, and CRP in diagnosing acute appendicitis demonstrated a sensitivity and negative predictive value of 99.33% and 98.46%, respectively, which were both significantly higher than those achieved with ultrasonography alone (P<0.05). However, the specificity of this combined approach was somewhat reduced (P<0.05). The positive predictive value for the combination of ultrasonography, PAS, and CRP was 73.40%, which was not significantly different from the positive predictive value of ultrasonography alone (P>0.05, refer to Table 5).

Table 5: Value of combination of ultrasonography and CRP in the diagnosis of acute complicated appendicitis

Diagnostic methods	Sensitivity(%)	Specificity (%)	Positive predictive value(%)	Negative predictive value(%)	Youden index
Ultrasonography alone	75.00 (84/112)	91.67 (143/156)	86.60 (84/97)	83.63 (143/171)	0.667
Combination of ultrasonography and CRP	98.21 (110/112)	77.56 (121/156)	75.86 (110/146)	98.37 (121/123)	0.862
χ - value	10.539	1.927	0.004*	6.082	
P- value	0.001*	0.165	0.950	0.014*	
* Means P-value < 0.05					

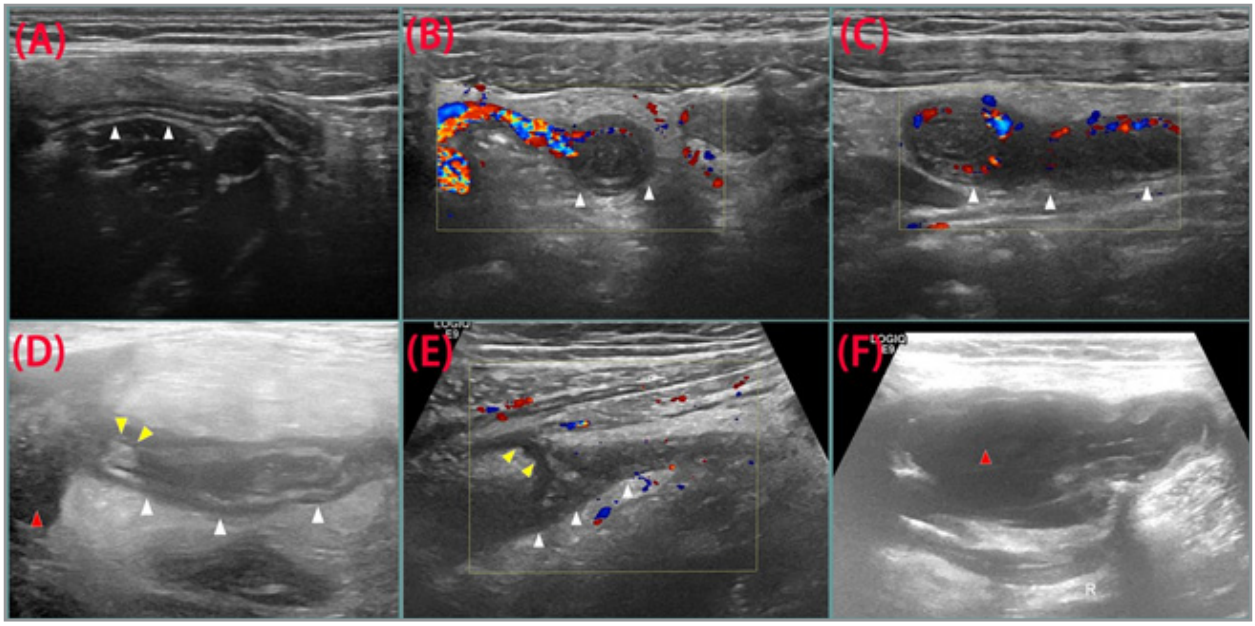

Figure 3: Ultrasonographic manifestations of various types of acute appendicitis

(A), Ultrasonography of simple appendicitis (longitudinal section): the white arrow, clear hierarchical boundaries of appendiceal wall
(B) and (C), Ultrasonography of suppurative appendicitis (transverse section): the white arrow, appendiceal wall (C), Ultrasonography of suppurative appendicitis (longitudinal section): the white arrow, appendiceal wall.
(D), Ultrasonography of suppurative appendicitis with stercolith: yellow arrow, stercolith; white arrow, appendix ; red arrow, cecum
(E), Ultrasonography of gangrenous perforative appendicitis: red arrow, perforation; white arrow: appendix.
(F), Ultrasonography of gangrenous appendicitis with periappendiceal abscess: red arrow, appendiceal cavity

CRP and Ultrasonography for Diagnosing Acute Complicated Appendicitis

The receiver operating characteristic (ROC) curves for C-reactive protein (CRP) and ultrasonography in diagnosing acute complicated appendicitis were constructed (refer to Figure 2). The areas under the ROC curves (AUCs) were found to be 0.777 (95% confidence interval, 0.716-0.838) for CRP and 0.897 (95% CI, 0.857-0.937) for ultrasonography, both with P-values less than 0.05. Ultrasonography proved to be highly effective for excluding acute complicated appendicitis, with a sensitivity of 86.60%. In the case of acute complicated appendicitis, the combined use of ultrasonography and CRP yielded a sensitivity and negative predictive value of 98.21% and 98.37%, respectively, which were significantly superior to those obtained with ultrasonography alone (P < 0.05; refer to Table 6).

Table 6: Comparison of PAS and CRP in patients with acute appendicitis

Groups	No.	PAS(Mean ± SD)	CRP(Mean ± SD, mg/L)
A	38	6.95 ± 1.64	31.03 ± 9.65
B	61	7.28 ± 1.52	37.06 ± 8.15a
C	51	7.47 ± 1.67	38.83 ± 7.38a
χ -value		2.240	10.23
P- value		0.110	< 0.001*
A group simple appendicitis			
B,group suppurative appendicitis			
C,group gangrenous appendicitis			
a means compares with group A, P < 0.05			
* Means P-value < 0.05			

Analysis of Different Types of Acute Appendicitis

Within the three groups categorized by the severity of acute appendicitis, no significant difference was observed in the PAS (P>0.05). However, the CRP levels in children with acute complicated appendicitis were notably elevated in comparison to those with acute simple appendicitis (P<0.05; refer to Table 7). The diagnostic accuracy of ultrasonography varied according to the pathological types of appendicitis, with rates of 78.95% for acute simple appendicitis, 81.97% for acute suppurative appendicitis, and 92.16% for acute gangrenous appendicitis. Furthermore, a Kappa value of 0.888 (P<0.05) denoted excellent agreement between ultrasonography and pathological examination for children with varying pathological types of appendicitis (refer to Table 8).

Table 7: Comparison of ultrasonographic and pathological diagnosis of appendicitis in children (No.)

Pathological diagnosis	Ultrasonographic diagnosis		
	Simple appendicitis	Suppurative appendicitis	Gangrenous appendicitis
Simple appendicitis	30	2	0
Suppurative appendicitis	2	50	2
Gangrenous appendicitis	0	4	47
Consistency between ultrasonic and pathological examination: kappa = 0.888, P- value < 0.001.			

Table 8: Ultrasonic features of appendices

Classification of appendicitis	Diameter	Appendiceal wall	Lumen of the appendix
Normal appendix	Usually less than 6 mm	Clearly layered, no thicker than 2 mm	With or without small amount of fluid
Simple appendicitis	Diameter ≥ 6mm	Clear hierarchical boundaries	Small amount of fluid in the cavity
Suppurative appendicitis	Swollen, with the rough and fuzzy outline	"Target ring" sign transversely, "bilateral" sign lengthwise	Echoes of stercolith could be found

Discussion

The lumen of the appendix in children is prone to obstruction by bezoars or food debris, attributed to the narrow inner diameter and the propensity for the appendix to twist. When bacteria penetrate the appendiceal wall, inflammation ensues. Unlike in adults, achieving accurate diagnosis in pediatric patients can be challenging due to atypical symptoms, uncooperative behavior during physical examination, and the variability in the clinical presentation of the condition.

As the two most popular clinical scoring systems for use in children, the PAS and Alvarado score and widely studied and appreciated in excluding acute appendicitis [4]. And the American College of Emergency Physicians approved that application of the Alvarado score as a triage clinical prediction rules that can be applied to 'rule out' appendicitis at a score below 5 points (sensitivity 94% to 99%) while PAS has successfully detected cases of appendicitis due to its high diagnostic sensitivity [7, 14]. In this investigation, utilizing a cutoff value of 6 for the PAS, the sensitivity and specificity for diagnosing acute appendicitis were found to be 82.67% (124 out of 150 cases) and 89.83% (106 out of 118 cases), respectively.

When employing a more lenient cutoff value of 5, the sensitivity increased to 93.33% (140 out of 150 cases), while the specificity decreased to 60.17% (71 out of 118 cases). The observed sensitivity and specificity may not be optimal, primarily due to the presence of atypical clinical symptoms in some preschool-aged children, uncooperative behavior during physical examinations, and rapid fluctuations in the patients' conditions. These findings

align with those reported in Song's study [15]. Nonetheless, the PAS does not distinguish significantly between the pathological types of acute appendicitis. To enhance diagnostic accuracy, it may be necessary to complement PAS with additional serum markers and imaging studies.

Regarding the diagnostic efficacy of CRP for acute complicated appendicitis, this study established a cutoff value for CRP of 32.26 mg/mL, yielding a sensitivity of 79.46% (89 out of 112 patients) and a specificity of 79.49% (124 out of 156 patients). Previous research has indicated that CRP levels are positively correlated with the severity of appendicitis, a finding that aligns with the outcomes of our study [16].

Nonetheless, normal CRP levels do not conclusively exclude the possibility of acute appendicitis. Therefore, the diagnosis of appendicitis should be integrated with clinical assessment and the evaluation of other inflammatory markers [17]. The investigation of laboratory markers for the diagnosis of pediatric acute appendicitis is an active area of research, with some markers demonstrating established utility and others still under evaluation. For example, pentraxin-3 levels have been shown to be beneficial in diagnosing acute appendicitis, whereas the role of white cell distribution width is still a subject of ongoing debate [18, 19].

Ultrasonography is a crucial tool in the diagnosis and pathological classification of acute appendicitis. The integration of clinical decision rules with ultrasonography has led to a reduced reliance on computed tomography for evaluating suspected appendicitis [5]. As the understanding of the ultrasound features in patients with appendicitis has advanced, ultrasonography has emerged as a diagnostic modality with a value akin to computed tomography, which is typically used as a backup due to its radiation exposure and higher cost.

Ultrasonography should be considered the primary imaging technique for children and pregnant women for diagnosing appendicitis, except in cases where the examination is hindered by factors such as intestinal gas or patient obesity [20]. In the current study, ultrasonography demonstrated a sensitivity and specificity of 82.67% and 89.93%, respectively, for diagnosing acute appendicitis. The reported sensitivity of ultrasound in diagnosing appendicitis ranges from 69.2% to 92.0%, with specificities ranging between 81.0% and 97.0% [15].

The variability in the effectiveness of different studies for diagnosing appendicitis may be attributed to the diversity of pathological types and the selection of ultrasound signs. The different pathological types of appendicitis are intimately connected to complications, surgical timing, and prognosis; delayed intervention may result in severe outcomes, including perforation and infection spread. Ultrasonography can provide insights into the pathological types of appendicitis prior to surgery, aiding clinicians in decision-making.

It has been recognized as highly valuable in differentiating acute simple appendicitis from complicated appendicitis; however, there is a paucity of studies focusing on the correlation between ultrasound findings and pathological classifications of acute appendicitis in pediatric patients [21].

In this investigation, ultrasonography demonstrated varying diagnostic accuracies for distinct pathological types of appendicitis: 78.95% (30 out of 38 cases) for acute simple appendicitis, 81.97% (50 out of 61 cases) for acute suppurative appendicitis, and 92.16% (47 out of 51 cases) for acute gangrenous appendicitis.

The relatively high diagnostic accuracy for acute suppurative and gangrenous appendicitis may be attributed to ultrasonography's capability to detect inflammation over an extensive area and to a profound depth. Acute complicated appendicitis often presents with significant inflammatory exudate and deep, extensive ulcerations of the appendiceal wall. In some cases, the pathology involves the entire thickness of the appendix wall, with some instances also featuring periappendiceal abscesses.

The ultrasonographic features of acute complicated appendicitis typically include thickening of the appendix, enhancement of the tube wall, dilation of the lumen, presence of coproliths within the lumen, periapendicular effusion, and lymph node enlargement, which facilitate its identification. However, the diagnostic accuracy of ultrasound for acute simple appendicitis is comparatively lower (78.95%), as the inflammation in these cases is confined to the mucosa and submucosa.

This results in minimal inflammatory exudate, less pronounced thickening of the appendix wall, and difficulty distinguishing the appendix from the surrounding normal mesenteric tissues and intestinal echoes. In this study, despite the inherent limitations of each method, the combination of ultrasonography with the PAS and CRP assessments significantly improved the sensitivity for diagnosing acute appendicitis to 99.33% (149 out of 150 patients) and 98.21% (110 out of 112 patients) for acute complicated appendicitis, respectively. Such an approach can be highly beneficial for clinicians in making informed clinical decisions.

However, there are limitations to the present study. The primary constraints arise from its retrospective design, which introduces the potential for selection bias and reliance on existing data. These factors may affect the interpretation of the study's outcomes and limit the generalizability of the findings. To address these issues, future research should focus on prospective studies with larger and more diverse participant cohorts. Moreover, this study focused solely on pediatric patients presenting with acute abdominal pain and a confirmed diagnosis of appendicitis, which may have restricted the scope of the investigation. The sample size of 268 cases could be expanded, and multiple centers could be included to enhance the robustness of the findings.

In summary, the integration of the Pediatric Appendicitis Score (PAS), C-reactive protein (CRP), and ultrasonography proves to be a valuable approach in diagnosing acute appendicitis and differentiating between acute simple and complicated appendicitis. Ultrasonography, in particular, offers significant diagnostic utility and aids in identifying various pathological types of the condition.

Acknowledgements

We would like to express my sincere gratitude to the surgical nursing team, the pathology team, and the ultrasound department for their invaluable contributions to this research on the diagnosis of acute appendicitis. Their expertise and dedication have been instrumental in the successful completion of this study.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics Statement

The studies involving human participants were reviewed and approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author Contributions

HH and LYG: conception and design of the research. M-HL, WL CS, C-JW CP and J-HL: acquisition of data. J-BC and QF-S: analysis and interpretation of the data. YL and M-HL: statistical analysis. HH and M-HL: writing of the manuscript. J-YH: critical revision of the manuscript for intellectual content. All authors read and approved the final draft.

Funding

No authors received any funding resource.

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