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Biomarkers to estimate the probability of complicated appendicitis☆

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ABSTRACT

Background: The conventional paradigm that all children with appendicitis require an appendectomy is being challenged by the idea that some patients may be successfully managed non-operatively. The study aimed to determine if matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinase (TIMPs) are candidate biomarkers for estimating the probability of complicated appendicitis in pediatric patients.
Methods: The study was a single-institution, prospective cohort study. MMP and TIMP serum protein concentrations were measured in patients with suspected appendicitis. Three hundred and thirty-one patients were enrolled with appendicitis. Classification and Regression Tree (CART) analysis was used to determine the combination of candidate biomarkers that best predicted complicated appendicitis.
Results: The CART-generated decision tree for the derivation cohort included WBC count, MMP-8, MMP-9, MMP-12, TIMP-2, and TIMP-4 and had the following test characteristics for estimating the probability of complicated appendicitis (95% CI): AUC 0.86 (0.81–0.90); sensitivity 91% (83–96); specificity 61% (53–68); positive predictive value 58% (50–66); negative predictive value 92% (84–96); positive likelihood ratio (LR) 2.3 (1.9–2.8); and negative LR 0.15 (0.08–0.3).
Conclusions: MMPs and TIMPs have the potential to serve as biomarkers to estimate the probability of complicated appendicitis in pediatric patients. The multi-biomarker-based decision tree has test characteristics suggesting clinical utility for decision making.
Level of Evidence: Level II: Study of Diagnostic Test.

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Acute appendicitis is the most common condition requiring urgent abdominal surgery in children [1]. The longstanding rationale for appendectomy is the prevention of complications secondary to disease progression and perforation [2]. However, the traditional paradigm that all children with appendicitis require appendectomy is now being challenged by the concept that a subgroup of children with low risk of disease progression can be successfully managed non-operatively with antibiotics and supportive care alone [3–10]. A pilot study was performed to investigate the feasibility of this non-operative management approach [11]. A recent meta-analysis reported success rates of non-operative management to be 91% at 30-day follow-up and 73% at one-year follow-up [12]. Another prospective observational study using rigorous inclusion criteria demonstrated success rates of non-operative management to be 87% at 18-month follow-up [13].

Given the increasing interest in the non-operative management of appendicitis, it is crucial to reliably identify patients appropriate for non-operative management. A companion diagnostic test to reliably estimate which patients can be safely managed in this manner would be highly valuable. Our preclinical studies have identified matrix metalloproteinase-8 (MMP8) as an important mediator of intestinal injury in animal models [14,15]. We hypothesized that MMP-8, other matrix metalloproteinase family members, and endogenous tissue inhibitors of metalloproteinases (TIMPs) can serve as companion diagnostic biomarkers to estimate the probability of complicated appendicitis in pediatric patients.

1. Methods

1.1. Patient cohort and data collection

This is a single institution, prospective cohort study. The study conformed to good clinical practice guidelines and followed the recommendations of the Declaration of Helsinki. The study protocol was approved in September 2014 by the Cincinnati Children's Hospital

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Medical Center (CCHMC) Institutional Review Board (ID 2014–5392). The CCHMC IRB granted a waiver of informed consent.

Pediatric patients presenting to the emergency department with suspected appendicitis, and a serum sample obtained at the time of presentation were eligible for study enrollment. The primary outcome variable was complicated appendicitis as defined by an abscessed, perforated, or gangrenous appendix at the time of appendectomy. Both the operative report and pathology report were reviewed to determine a classification of complicated appendicitis. Study subjects were followed to determine their clinical course after evaluation for appendicitis.

1.2. Candidate biomarkers

MMP and TIMP serum protein concentrations were measured using a multiplex magnetic bead-based immunoassay designed by BIORAD Corporation (Hercules, CA, USA) and a Luminex 100/200 System (Luminex Corporation, Austin, TX, USA), according to the manufactures' specifications. The multiplex panel included measurements for MMP-1, -2, -3, -7, -8, -9, -10, -12, -13 and TIMP-1, -2, -3, -4.

1.3. Statistical analysis

The primary outcome variable for all modeling procedures was the presence of complicated appendicitis. Descriptive statistics and comparisons were conducted using SigmaStat Software (Systat Software, Inc., San Jose, CA, USA). Receiver operating characteristic curves were constructed to determine the ability of each candidate biomarker to estimate the probability of complicated appendicitis. Classification and Regression Tree (CART) methodology (Salford Predictive Modeler v8.0, Salford Systems, San Diego, CA) was used to determine the combination of candidate biomarkers that best predicted complicated appendicitis [16]. All 13 candidate biomarkers, as well as white blood cell (WBC) count were considered as predictor variables in the CART analysis. Weighting of cases or introducing a cost for incorrect predictions was not used in the modeling procedures. The derivation cohort was randomly selected to include 80% of the study cohort; the test cohort consisted of the remaining 20%. Performance of the derivation and test cohort decision trees are reported using diagnostic test statistics with 95% confidence intervals. Statistical significance was defined as $p < 0.05$.

2. Results

2.1. Derivation of the decision tree

Five hundred and eighty-seven subjects who presented to the emergency department with suspected appendicitis were enrolled in the study. Among these, 331 had surgically confirmed appendicitis, and 125 (38%) of these had complicated appendicitis. The primary analysis is based on these 331 subjects with appendicitis. We randomly selected 80% of these subjects ($n = 265$) for the derivation cohort. The demographic and clinical characteristics of the study subjects in the derivation cohort are depicted in Table 1. Among the individual candidate biomarkers, MMP-8 had the greatest area under the curve (AUC = 0.68; $p < 0.001$) for estimating the probability of complicated appendicitis. MMP-9, MMP-10, MMP-12, MMP-13, TIMP-2 and TIMP-4 also had AUCs approaching 0.60 and p values ≤0.05 (Table 2). WBC count alone had an AUC of 0.77, $p < 0.001$.

Fig. 1 shows the derived decision tree. Maximum accuracy for estimating the risk of complicated appendicitis was attained with 5 of the 13 candidate biomarkers: MMP-8, −9, −12, TIMP2, and TIMP4. There were three low-risk terminal nodes (≤ 20.8% risk of complicated appendicitis; nodes 1, 3, and 7), two moderate risk terminal nodes (27.5–47.6% risk of complicated appendicitis; nodes 2 and 4), and three high-risk terminal nodes (59.1–90.0% risk of complicated appendicitis; nodes 5, 6, and 8). Table 3 shows the diagnostic test characteristics of the decision tree for the derivation cohort, wherein subjects in the low-risk terminal nodes are classified as predicted to not have complicated appendicitis, and subjects in the moderate and high risk terminal nodes are classified as predicted to have complicated appendicitis.

Table 1
Demographic and clinical characteristics of the derivation and test cohorts.

	Derivation cohort (n = 265)		Test cohort (n = 66)	
	Uncomplicated	Complicated	Uncomplicated	Complicated
N (% total)	165 (62)	100 (38)	41 (62)	25 (38)
# Males (%)	104 (63)	65 (65)	21 (51)	13 (52)
# Females (%)	61 (37)	35 (35)	20 (49)	12 (48)
Median age years (range)	11	10	10	10
[IQR]	[9–14]	[7–12.5]	[8–13]	[8–12]
# for race (%)				
Caucasian	129 (78)	82 (82)	35 (85)	19 (76)
African American	17 (10)	12 (12)	2 (5)	2 (8)
Other	19 (12)	6 (6)	4 (10)	4 (16)
# with temp >38 °C (%)	8 (5)	37 (37)	6 (15)	9 (36)
# with vomiting (%)	85 (52)	69 (69)	20 (49)	15 (60)

2.2. Testing the decision tree

The demographic and clinical characteristics of the study subjects in the test cohort ($n = 66$) are depicted in Table 1. The test cohort subjects were classified based on the derived decision tree, without any modifications. Table 4 shows the diagnostic test characteristics of the decision tree in the test cohort.

2.3. Secondary considerations

Fig. 2 compares the receiver operating characteristic (ROC) curves for the decision tree and WBC count alone for all subjects in the derivation and test cohorts. The AUC of the decision tree (0.86; 95% CI: 0.82 to 0.90) was superior to that of WBC count alone (0.77; 95% CI: 0.72 to 0.82; $p = 0.0003$) for estimating the risk of complicated appendicitis.

Ten of the initial 587subjects enrolled were taken to the operating room for an appendectomy but were found to have a normal appendix. All of these patients fell into low-risk terminal nodes (nodes 1, 3, or 7). The decision tree would have identified these subjects as having a low probability of complicated appendicitis and they might have avoided an operation under a non-operative management protocol.

Table 2
Areas under the receiver operating curves for individual biomarkers.

Biomarker	AUC	95% CI	p value
Higher value associated with complicated appendicitis			
MMP1	0.51	0.50–0.58	0.667
MMP2	0.52	0.46–0.59	0.502
MMP3	0.56	0.50–0.62	0.071
MMP7	0.51	0.50–0.58	0.658
MMP8	0.68	0.63–0.74	<0.001
MMP9	0.62	0.56–0.69	<0.001
MMP10	0.59	0.53–0.65	0.006
MMP13	0.57	0.51–0.63	0.031
TIMP4	0.58	0.52–0.64	0.012
WBC	0.77	0.71–0.84	<0.001
Lower value associated with complicated appendicitis			
MMP12	0.57	0.51–0.64	0.025
TIMP1	0.54	0.48–0.60	0.223
TIMP2	0.61	0.55–0.67	<0.001
TIMP3	0.52	0.45–0.58	0.618