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Diagnostic Value of Pancreatic Stone Protein in comparison to White Cell Count and C-Reactive Protein in the Diagnosis of Acute Appendicitis – A Prospective Multicenter Diagnostic Accuracy Trial

Dimitri Aristotle Raptis[1], Dilmurodjon Eshmuminov [1], Christoph Tschuor[1], Perparim Limani[1], Thomas Neff[10], Theresia Reding [1], Florian Martens[2], Pietro Renzulli[2], Jeannette D Widmer[3], Markus K Muller[3], Marcel Schibli[4], Roman Lässker[4], Daniel C Steinemann[5], Eliane Angst[6,7], Martin Bosch[6,8], Kyriakos Psarianos[9], Dimitrios Lytras[9] and Rolf Graf[1*]

[1]Department of Surgery and Transplantation, University Hospital Zurich, Switzerland

[2]Department of Surgery, Cantonal Hospital Muensterlingen, Switzerland

[3]Department of Surgery, Cantonal Hospital Frauenfeld, Switzerland

[4]Department of Surgery, Herisau Hospital, Appenzell, Switzerland

[5]Department of Surgery, Cantonal Hospital Baselland, Location Bruderholz, Switzerland

[6]Department of Surgery, Cantonal Hospital Schaffhausen, Switzerland

[7]Department of Surgery, Cantonal Hospital Glarus, Switzerland

[8]Department of Surgery, Hospital Uznach, Switzerland

[9]Department of Surgery, General Hospital Volos, Greece

[10]Department of Anesthesiology, Intensive Care & Pain Medicine, Cantonal Hospital Muensterlingen, Switzerland

[*]**Corresponding author:** Rolf Graf, Department of Surgery and Transplantation, University Hospital Zurich, Raemistrasse 100, CH-8091, Switzerland. Email: rolf.graf@usz.ch

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Abstract

Background: Acute appendicitis is a common cause of acute abdominal pain and remains a diagnostic challenge. In the search for improved diagnostic characterization, the authors aimed at determining the diagnostic accuracy of Pancreatic Stone Protein (PSP), in predicting acute appendicitis.

Methods: The "PSP Appendix Trial" is a prospective, international, multicenter, diagnostic accuracy study, including consecutive patients with a clinical suspicion of acute appendicitis. The index test was PSP and its diagnostic accuracy was compared to White Blood Cell Count (WBC), C-Reactive Protein (CRP), the Alvarado score, Ultrasound (US) and Computer Tomography (CT) for appendicitis evident on histology. (NCT01610193)

Results: A total of 357 patients presented with clinical suspicion of acute appendicitis, of whom 301 (84%) were admitted to the hospital, 230 (64%) were operated, and 194 (54%) were diagnosed with acute appendicitis on histology. Of all tests, low PSP values of <40ng/ml had the highest accuracy (84%, p<0.001) in predicting appendicitis on histology when compared to the Alvarado score >6 (60%, p=0.132), WBC > 10G/l (74%, p=0.250), CRP >5 mg/l (67%, p=0.299), ultrasound (67%, p=0.023) and CT (83%, p= N/A).

Conclusion: In contrast with our initial hypothesis, higher PSP levels were associated with the likelihood of having complex diagnosis, more complex than acute appendicitis. Using a cut-off value of 40ng/ml (when <40/positive), PSP had the highest accuracy in predicting appendicitis on histology when compared to the Alvarado score, CRP, US and CT. Integration of PSP in the differential diagnosis of right iliac fossa pain may provide useful information to decide whether appendicitis was accompanied by surgically more complicated situations. Low values were an indicator for straight surgical appendicitis.

Keywords: Appendicitis; PSP

Abbreviations: AUC: Area Under the Curve; CCI: Comprehensive Complications Index®; CRP: C-Reactive Protein; CT: Computed Tomography; ED: Emergency Department; PSP: Pancreatic Stone Protein; RLQ: Right Lower Quadrant; WBC: White Blood Cell Count; USS: Ultrasound Scan

Introduction

Appendicitis is a common condition that necessitates urgent surgery. It may present with a variety of clinical manifestations often overlapping with other common clinical entities of abdominal pain, while delay in diagnosis increases significantly the morbidity rates resulting to even potentially life threating complications if left untreated on time. Despite diagnostic and therapeutic advancement in recent years, the diagnosis of acute appendicitis remains mainly clinical [1]. White Blood Cell count (WBC) and C-Reactive Protein (CRP) vary significantly depending on the duration of symptoms. Based on current diagnostic strategies, a misdiagnosis rate of 5-15% is still reported [1,2].

In search for novel inflammatory markers, the authors came across Pancreatic Stone Protein (PSP), a secretory protein produced predominantly in the pancreas and the gut. There is evidence from experimental and clinical trials that the levels of PSP in the blood increase in the presence of inflammation or infection [3,4]. PSP was shown in a population of patients with sepsis-related complications to have a high diagnostic accuracy in discriminating the severity of peritonitis and predicting mortality in the Intensive Care Unit (ICU) [5]. Furthermore, PSP was identified as a biomarker related to organ failure and outcome in patients with ventilator associated pneumonia [4]. However, it is unknown whether PSP is similarly superior to WBC or CRP in predicting appendicitis. The objective of this trial is to determine the diagnostic accuracy of PSP in predicting acute appendicitis and to compare PSP with other established markers used in the diagnostic work-up.

Methods

Study Design

The "PSP Appendix Trial" was a prospective, multi-center, diagnostic accuracy cohort study designed to assess the value of PSP in the diagnostic workup of acute appendicitis. The local ethics committee has approved the study protocol. The study protocol was registered at ClinicalTrials.gov [NCT01610193] as well as published in advance [6]. The authors used the STARD checklist [7] for reporting the findings.

Participants

All consecutive adult patients who presented with right iliac fossa pain and a clinical suspicion of acute appendicitis, qualified for study inclusion. This study was designed to be a pragmatic trial and thus recruitment was based upon the clinical judgment of the surgeons. All patients who fulfilled the inclusion criteria but refused study participation as well as patients who were not primarily detected as eligible patients were excluded from the study and were recorded. Study recruitment started on the 28th of February 2012 and ended on the 17th of November, 2015. Table 1 lists all participating centers.

Patient characteristics	All patients (n= 357)	Appendicitis (n= 194)	No Appendicitis (n=163)	OR (95% CI)	p value
Age in years, median (i.q.r)	31 (24-47)	32 (24-46)	30 (24-47)	-	0.867
Gender, n (%)					
Male	167 (46.8)	106 (54.6)	61 (37.4)	2.0 (1.3-3.2)	**0.001**
Female	190 (53.2)	88 (45.4)	102 (62.6)		
Ethnicity, n (%)					
Caucasian	341 (95.5)	186 (95.9)	155 (95.1)	-	0.750
Asian	13 (3.6)	6 (3.1)	7 (4.3)	-	-
African	1 (0.3%)	1 (0.1)	0 (0.0)	-	-
Other	2 (0.6)	1 (0.1)	1 (0.1)	-	-
Comorbidities, n (%)					
Yes	84 (23.5)	44 (22.7)	40 (24.5)	0.9 (0.5-1.5)	0.708

Patient characteristics	All patients (n= 357)	Appendicitis (n= 194)	No Appendicitis (n=163)	OR (95% CI)	p value
No	273 (76.5)	150 (77.3)	123 (75.5)	-	-
Institution, n (%)					
Cantonal Hospital Munsterlingen, Switzerland	125 (35.0)	54 (27.8)	71 (43.6)	-	**< 0.001**
Cantonal Hospital Frauenfeld, Switzerland	68 (19.0)	36 (18.6)	32 (19.6)	-	-
University Hospital Zurich, Switzerland	61 (17.1)	41 (21.1)	20 (12.3)	-	-
Herisau Hospital Appenzell, Switzerland	34 (9.5)	19 (9.8)	15 (9.2)	-	-
Bruderholz Hospital, Switzerland	30 (8.4)	12 (6.2)	18 (11.0)	-	-
Cantonal Hospital Schaffhausen, Switzerland	23 (6.4)	18 (9.3)	5 (3.1)	-	-
General Hospital Volos, Greece	16 (4.5)	14 (7.2)	2 (1.2)	-	-
Hours from symptoms to presentation at the ER, median (i.q.r)	23 (10-48)	19 (12-36)	24 (9-72)	-	0.050
Abdominal pain that migrates to the right iliac fossa					
Yes	262 (73A)	149 (77.2)	113 (69.3)	1.5 (0.9-2.5)	0.117
No	94 (26.3)	44 (22.8)	50 (30.7)	-	-
Missing	1 (0.3)			-	-
Anorexia					
Yes	222 (62.2)	130 (67.0)	92 (56.4)	1.6 (1.0-2.5)	**0.049**
No	135 (37.8)	64 (33.0)	71 (43.6)	-	-
Nausea or vomiting					
Yes	184 (51.5)	101 (52.3)	83 (51.2)	1.0 (0.7-1.6)	0.915
No	171 (47.9)	92 (47.7)	79 (48.8)		-
Missing	2 (0.6)			-	-
Tenderness in right iliac fossa					
Yes	323 (90.5)	179 (92.3)	144 (88.3)	1.6 (0.8-3.5)	0.277
No	34 (9.5)	15 (7.7)	19 (11.7)	-	-
Rebound tenderness					
Yes	199 (55.7)	140 (72.2)	59 (36.2)	4.6 (2.9-7.3)	**< 0.001**
No	158 (44.3)	54 (27.8)	104 (63.8)		
Raised temperature (≥37.3 ˚C)					
Yes	67 (18.8)	40 (20.6)	27 (16.6)	1.3 (0.7-2.3)	0.344
No	290 (81.2)	154 (79.4)	136 (83.4)	-	-
Hospital admission					
Admitted to the hospital ward	301 (84.3)	194 (100)	107 (65.6)	-	**< 0.001**
Discharged home directly from the ER	56 (15.7)	0 (0.0)	56 (34.4)	-	
Re-attendance to ER, n (%)	19 (5.3)	7 (3.6)	12 (7.4)	0.5 (0.2-1.3)	0.155

Table 1: Baseline demographic and clinical characteristics of participants.

Test Methods

The index test was PSP value, a peripheral blood test obtained from all patients with a clinical suspicion of appendicitis upon attendance at the Emergency Department (ED). At the same time, blood samples were obtained among others, for WBC and CRP, as routinely performed in all patients with abdominal pain. Additionally, the clinical parameters for the Alvarado likelihood of appendicitis score [8] (Table 2) were collected, including recording of relevant symptoms related to appendicitis. Abdominal Ultrasound Scan (USS) and Computed Tomography (CT) were freely performed according to the routine decision of the investigators. Imaging according to the final reports were recorded and used for further diagnostic accuracy analysis.

The Alvarado likelihood of appendicitis, n (%)	All patients (n= 357)	Appendicitis	No Appendicitis	OR (95% CI)	p value
Unlikely appendicitis	83 (23.2)	23 (27.7)	60 (72.3)	-	**< 0.001**
Possible appendicitis	118 (33.1)	53 (44.9)	65 (55.1)	-	-
Likely appendicitis	113 (31.7)	86 (76.1)	27 (23,9)	-	-
Very likely appendicitis	43 (12.0)	32 (74.4)	11(25.6)	-	-
Alvarado score, median (i.q.r.)	6 (5-8)	7 (6-8)	5 (4-6)	-	**< 0.001**
Alvarado score grouped					
Alvarado score 7-10	156 (44)	118 (75,6)	38 (24.4)	5.1 (3.2-8.1)	**< 0.001**
Alvarado score 0.6	201 (56)	76 (37.8)	125 (62.2)	-	

Table 2: The Alvarado score and likelihood of appendicitis categories.

In a first analysis (A) including all patients with clinical suspicion of appendicitis, the reference standard was the "diagnosis of appendicitis" or "no appendicitis" (dichotomous variable) upon discharge of the patient, judged either directly at admittance from the emergency room, or during the entire course of the hospitalization upon discharge. The diagnosis of appendicitis was based either on clinical or imaging criteria, if discharged from the emergency room or the hospital ward. Thus, all patients, even those who did not undergo surgery, had an assessment with regard to the diagnosis of appendicitis and an initial analysis of PSP in the emergency room setting at hospital admittance (Figure 1). In a second analysis (B), focusing on the primary endpoint of this study, the reference standard was the presence or absence of appendicitis based on the definitive histopathological report (Figure 1). Thus, this cohort included only patients who had the appendix removed.

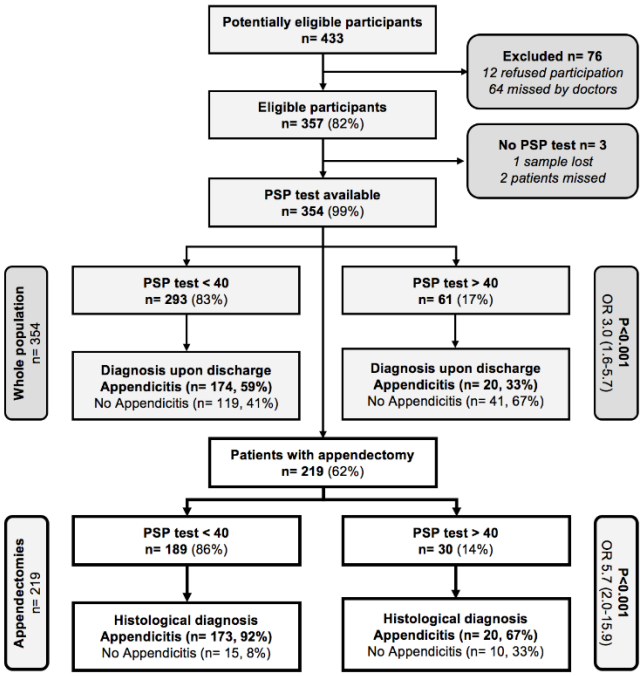

Figure 1: Patient flow.

The optimal cut-off values for test positivity for PSP, WBC and CRP were determined by performing ROC curve analysis and based on the Youden's index, known to give equal weight to sensitivity and specificity, as previously described [5]. The investigators were blinded to the PSP values obtained from the patients. However, WBC and CRP values were performed at the local laboratories and were immediately available to the investigators. The blood samples obtained for PSP measurements were separately stored and analyzed using the ELISA technique [5].

Data Collection

The assessment for patient recruitment started as soon as the patient entered ED. If there was a clinical suspicion for appendicitis, the patient was further assessed for eligibility to participate in the study. Once a patient was found to meet the study inclusion criteria, the patient entered immediately the trial before any other investigations were ordered. Data collection included basic demographics, clinical status, laboratory values, the use and the findings of diagnostic imaging, whether the patients were discharged from ED at home, admitted to the ward or referred to another medical team. During

hospitalization, data regarding medical or surgical treatment, the operation type and findings, and any complications using the Clavien-Dindo Classification of surgical complications [9] as well as the Comprehensive Complications Index® (CCI®) [10,11] were recorded. Finally, the histopathology reports of operated patients were also recorded. Data capture in this multicenter trial occurred with the use of a specially designed (by DAR), encrypted and password protected, electronic Clinical Trial Management System (CTMS) [https://www.PSPtrial.com], as previously described [12].

Sample size

Due to the lack of data in the literature on PSP levels in patients with appendicitis, a sample size calculation was performed to ensure an adequate event rate, i.e. appendicitis on histology in patients that were operated, of over 100 patients. Assuming a negative appendectomy rate, i.e. appendices surgically removed but normal on histopathology, of 15%, hence 85% of patients with appendicitis, an effect size difference of 0.80, an alpha error of 0.05, a power of 80%, and an allocation ratio of 0.185, a total of 130 patients undergoing appendectomy were needed. Thus, assuming 20% of patients admitted but not operated and 20% of patients presenting to the emergency room but discharged home, a total of 204 patients were needed to be recruited for the study. To adjust for loss to follow up or missing values, at least 245 patients were estimated for study inclusion, as previously reported [6].

Statistical Analysis

Continuous variables were compared with the Student t, Mann–Whitney U, one-way ANOVA, and Kruskal-Wallis tests, where appropriate. Differences among proportions derived from categorical data were compared using the Fischer's Exact or the Pearson $\chi 2$ tests, where appropriate. All p values were two-sided and considered statistically significant if $p \leq 0.05$. Sensitivity, specificity, accuracy, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (PLR), Negative Likelihood Ratio (NLR), Yuden's Index (YI), diagnostic Odds Ratio (OR), and the Receiver Operator Characteristic (ROC) curve were also calculated. For better interpretation of the different diagnostic accuracy results, the arbitrary scale was used with a value of >0.81 being very high, 0.61 - 0.80 high, 0.41 - 0.60 moderate, 0.21 - 0.40 low, and 0.01 - 0.20 very low. Data are presented as mean (SD), median (i.q.r.) and proportions (%) with odds ratios (95% CI), where appropriate. Statistical analysis was performed using R version 3.3.2 (R Core Team, GNU GPL v2 License), R Studio version 1.0.44 (R Studio, Inc. GNU Affero General Public License v3, Boston, MA, 2016) with the graphical user interface rBiostatistics.com alpha version (rBiostatistics.com, Zurich, Switzerland, 2016) [13].

Results

What were the characteristics of the patients?

A total of 357 eligible patients (167 males, 190 females) presented at the emergency room with abdominal pain and clinical suspicion of appendicitis. Tables 1,2 list their clinical

characteristics. Blood samples were obtained from all patients with a mean WBC of 12.5±4.3x10^9/l and a mean CRP of 43.9±53.5 mg/l. The mean Pancreatic Stone Protein (PSP) values, was 37±67ng/ml Table 3 and this observation was not available to the managing doctors.

	All patients (n= 357)	Appendicitis	No Appendicitis	OR (95% CI)	p value
White blood cell count (WBC)					
WBC, mean (SD)	12.5 (4.3)	13 (4.0)	11(4.0)	-	< 0.001
WBC, median (i.q.r.)	12 (10-15)	13 (11-16)	11(9-14)	-	< 0.001
WBC > 10 grouped	237 (66)	154 (65.0)	83 (35.0)	3.7 (2.3-5.9)	< 0.001
WBC≤10 grouped	120 (34)	40 (33.3)	80 (66.7)	-	-
C-reactive protein (CRP)					
CRP, mean (SD)	43.9 (53.5)	47 (52)	40 (56)	-	0.248
CRP, median (i.q.r.)	25 (4-61)	31(8-66)	12 (2-57)	-	0.001
CRP > 5 grouped	227 (63.6)	155 (69.3)	72 (31.7)	3.3 (2.0-5.4)	< 0.001
CRP≤5 grouped	93 (26)	37 (39.8)	56 (60.2)	-	-
Missing CRP	37 (10)	-	-	-	-
Pancreatic stone protein (PSP)					
PSP, mean (SD)	37 (67)	25.4 (32.9)	51.1 (91.2)	-	< 0.001
PSP, median (i.q.r.)	18 (13-29)	18 (13-27)	20 (13-42)	-	0.097
PSP > 40 grouped	61 (17.1)	20 (32.8)	41 (67.2)	0.3 (0.2-0.6)	< 0.001
PSP≤40 grouped	293 (82.0)	174 (59.4)	119 (40.6)	3.0 (1.7-5.4)	-
Missing PSP	3 (1)	-	-	-	-

Table 3: Laboratory values.

USS was performed in 263 (73.7%) and CT in 53 (14.8%) patients. In 29 (9%) patients, USS was not conclusive, and therefore followed by an additional CT. Majority of USS were performed in women (150/171, 88%) as compared to men (113/152, 74%) (OR 2.5 (1.3-4.7), p=0.003), while CT were more equally distributed between genders (29/171, 17%, vs. 24/152, 16%, (OR 2.6 (1.3-4.66), p=0.881). Of all 357 patients who presented at the emergency room, 56 (16%) were discharged directly after initial assessment, while 301 (84%) were admitted to the hospital ward. Re-attendance to the emergency room later than one day after primary discharge at home occurred in 19 (5%) patients, 7 of whom were diagnosed subsequently with appendicitis after re-evaluation. The final diagnoses of the 56 patients upon discharge directly from the emergency room on the same day are listed in Supplementary Table S1.

Diagnosis upon discharge	Discharged patients, n=56, (%)
Gastroenteritis	17 (30.4)
Non-specific abdominal pain	9 (16.1)
Constipation	9 (16.1)
Enterocolitis	6 (10.7)
Musculoskeletal pain	3 (5.4)
Urinary tract infection	3 (5.4)
Ruptured ovarian cyst	2 (3.6)
Acute diverticulitis	1 (1.8)
Adnexitis	1 (1.8)
Endometriosis	1 (1.8)
Inguinal hernia	1 (1.8)
Pregnancy	1 (1.8)
Reflux	1 (1.8)
Teratoma	1 (1.8)

Supplementary Table S1: Diagnosis upon discharge directly from the emergency room.

How were the hospitalized patients managed?

Hospitalization was defined as any patient being admitted from the emergency room directly to the theatre room or to the hospital ward. Of the 301 patients admitted, 230 (76%) received surgery, while 127 (32%) were treated conservatively. Supplementary Table S2 lists the operation characteristics of the participants. Of all 230 operated patients, diagnosis of acute appendicitis was confirmed intraoperatively and on the histopathology report in 194 (84%) patients (194/357, 54% for the whole cohort). Furthermore, of the 230 operated patients, 219 (95%) had their appendix removed while the remaining 11 (5%) received another operation without appendectomy. In these 11 patients, appendicitis was excluded preoperatively with CT and the indication for surgery was not appendicitis. Of the 219 patients who received an appendectomy, 194 (89%) had appendicitis confirmed based on histology of the removed specimen while the remaining 25 had no evidence of appendicitis. Thus, the negative appendectomy (i.e. appendectomy normal in the histopathology report) rate was 11% (25/219) in our series Supplementary Table S3. Figure 1 illustrates the patient flow. Supplementary Table S4 indicates the different types of diagnoses of patients upon discharge from the hospital. Postoperative morbidity as graded by the Clavien-Dindo classification and the quantified by the Comprehensive Complication Index® (CCI®) is reported in Supplementary Table S5.

Operation characteristics	All patients, n= 357 (%)
Operated	
Yes	230 (64.4)
No	127 (35.6)
Operation type	
Laparoscopic appendectomy	180 (78.6)
Laparoscopic converted to midline laparotomy	3 (1.3)
Open appendectomy Mc Burney incision	24 (10.5)
Open appendectomy midline laparotomy	11 (4.8)
Other	11 (4,8)
Intraoperative finding	
Normal appendix	21 (9.3)
Thickened appendix	133 (58.6)
Gangrenous appendix	20 (8.8)
Perforated appendix	37 (16.3)
Periappendicular abscess	4 (1.8)
Other	12 (5.3)

Supplementary Table S2: Operation characteristics.

Histological diagnosis	Operated patients, n= 230 (%)
Appendicitis on histology, n (%)	
Yes	197 (90.4)
No	21 (9.6)
Other specimen	12 (NA)
Histological diagnosis	
Normal appendix	19 (8.7)
Acute appendicitis	48 (22.0)
Gangrenous appendix	22 (10.1)
Phlegmonous appendix	82 (37.6)
Perforated appendix	39 (17.9)
Periappendicular abscess	5 (2.3)
Neurogenic appendicopathy	3 (1.0)

Supplementary Table S3: Histological diagnosis.

Diagnosis upon discharge	All patients (n= 357), %
Acute appendicitis	128 (35.8)
Non-specific abdominal pain	42 (11.8)
Perforated appendicitis	40 (11.2)
Gastroenteritis	35 (9.8)
Gangrenous appendicitis	22 (6.2)
Constipation	15 (4.2)
Enterocolitis	12 (3.4)
Acute diverticulitis	8 (2,2)
Ruptured ovarian cyst	7 (2.0)
Urinary tract infection	6 (1.7)
Acute cholecystitis	5 (1.4)
Periappendicular abscess	4 (1.1)
Endometriosis	3 (0.8)
Musculoskeletal pain	3 (0.8)
Adnexitis	2 (0.6)
Cecum tumor	2 (0.6)
Neurogenic appendicopathy	2 (0.6)
Ovarian abscess	2 (0.6)
Pancreatitis	2 (0.6)
Carcinoid of the appendix	1 (0.3)
Mucinous neoplasia of the appendix	1 (0.3)
NET of the appendix	1 (0.3)
Crohn's disease	1 (0.3)
Dysmenorrhea	1 (0.3)
Echinococcus of the ovary	1 (0.3)
Ileus	1 (0.3)
Infected renal cyst	1 (0.3)

Inguinal hernia	1 (0.3)
Meckel diverticulitis	1 (0.3)
Ovulation pain	1 (0.3)
Perforated duodenal ulcer	1 (0.3)
Pregnancy	1 (0.3)
Pyelonephritis	1 (0.3)
Reflux	1 (0.3)
Teratoma	1 (0.3)
Urolithiasis	1 (0.3)

Supplementary Table S4: Diagnosis upon discharge from the emergency room or hospital ward.

Postoperative Complications	Operated patients, n= 357, (%)
Any complication	
Yes	18 (7.8)
No	212 (92.2)
Clavien-Dindo complication grade	
No complication	212 (92.2)
Grade I	10 (4.3)
Grade II	3 (1.3)
Grade IIIa	4 (1.7)
Grade IIIb	1 (0.4)
Grade IVa	0
Grade Ivb	0
Grade V (death)	0
Comprehensive Complications Index (CCI)	
Mean (SD)	1.1 (4.6)

Supplementary Table S5: Postoperative Complications.

What was the diagnostic value of the Alvarado likelihood score in the diagnosis of acute appendicitis?

The Alvarado likelihood score for acute appendicitis, including common symptoms, signs, WBC and neutrophils was completed in all patients as soon as they were enrolled to the trial (Table 2). Of all 357 patients, 201 (56%) had a low (score 0-6) while 156 (44%) had a high likelihood of acute appendicitis score (score 7-10). In the 194 patients with acute appendicitis, 118 (61%) had a high Alvarado score while 76 (39%) had a low score. In the 163 patients without appendicitis, the Alvarado score was high in 38 (23%) patients and low in the remaining 125 (77%) patients (OR 5.1, 95% CI 3.2-8.1, p<0.001). The diagnostic accuracy based on the ROC curve analysis of the Alvarado score (low vs. high) is indicated in Table 5, with an accuracy of 68% (p<0.001).

What was the diagnostic value of WBC, CRP and PSP in the diagnosis of appendicitis?

Blood for measurement of WBC, CRP and PSP was obtained from all 357 patients, early during their attendance at the emergency room (Table 3). The mean WBC count in patients with acute appendicitis was 13±4.3x10^9/l and in patients without appendicitis (or any other condition) was 11±4.1x10^9/l (p<0.001). The mean CRP in patients with appendicitis was 47±52 mg/l and those without (or any other condition) was 40±56mg/l (p=0.248). The mean PSP in patients with appendicitis was 25±33 ng/ml while in patients without appendicitis (or any other condition) was surprisingly higher, with a mean of 51±91ng/ml (p<0.001) (Figure 2). Thus, based on these findings, the authors decided to change the test positivity when PSP was lower and negative when higher. The accuracy for acute appendicitis was moderate regarding WBC>10x10^9/l, CRP>5 mg/l and PSP<40 ng/ml, with 66%, 66% and 61%, respectively Table 5, Figure 3.

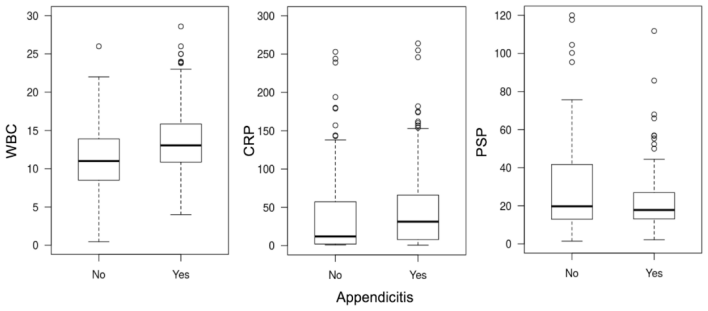

Figure 2: Laboratory values in patients with and without appendicitis upon discharge.

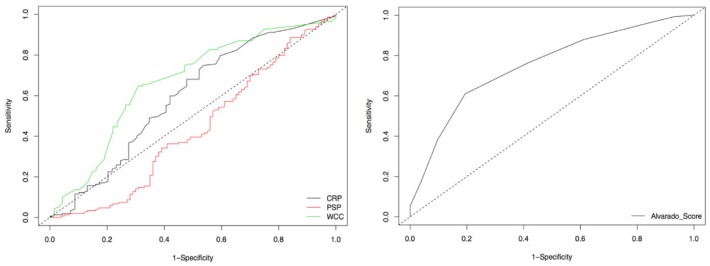

Figure 3: ROC Curve analysis for laboratory values and the Alvarado score for appendicitis.

What was the diagnostic value of USS and CT in the diagnosis of appendicitis?

The use of diagnostic imaging methods, USS and CT, and their respective findings are reported in Table 4. The accuracy of ultrasound performed by radiologists at the emergency room for the diagnosis acute appendicitis was 77%, with a sensitivity of 89% and specificity of 66% (p<0.001 for the Area Under the Curve

(AUC)). The accuracy of CT was as expected higher at 91% as compared to ultrasound, with a sensitivity of 83% and specificity as high as 100% (p<0.001 for the AUC) (Table 5).

	All patients (n= 357)	Appendicitis	No Appendicitis	OR (95% CI)	p value
Abdominal ultrasound performed, n (%)					
Yes	263 (81.4)	152 (57,8)	111 (42,2)	0.64 (0.35-1.15)	0.146
No	94 (18.6)	41 (68.3)	19 (31.7)		-
Appendix visualized					
Yes	158 (60,3)	122 (77,2)	36 (22.8)	8.76 (4.97-15.46)	**<0.001**
No	104 (39.7)	29 (27.9)	75 (72.1)		-
Appendix thickened					
Yes	101 (42,1)	89 (88.1)	12 (11.9)	9.48 (4.76-18.90)	**<0.001**
No	139 (57.9)	61 (43.9)	78 (56.1)		-
Free fluid detected					
Yes	78 (29.9)	49 (62.8)	29 (37.2)	1.34 (0.78-2.31)	0.178
No	183 (70.1)	102 (55.7)	81 (44.3)		-
Evidence of acute appendicitis					
Yes	115 (43.9)	103 (89.6)	12 (10.4)	17.17 (8.62-34.20)	**<0.001**
No	147 (56.1)	49 (33.3)	98 (66.7)	-	-
CT scan performed, n (%)					
Yes	53 (14.8)	30 (65.5)	23 (43.4)	0.86 (0.47-15.3)	0.358
No	270 (83.6)	163 (60.4)	107 (39.6)		-
Appendix visualized					
Yes	46 (85.2)	30 (65.2)	16 (34.8)	-	**0.001**
No	8 (14.8)	0 (0)	8 (100.0)	-	-
Appendix wall thickened					
Yes	24 (44.4)	24 (100,0)	0 (0.0)	-	**<0.001**
No	30 (55.6)	6 (20.0)	24 (80.0)	-	-

	All patients (n= 357)	Appendicitis	No Appendicitis	OR (95% CI)	p value
Appendix dilatation					
Yes	20 (37.0)	19 (95.0)	1(5,0)	39.7 (4.70-336.11)	**<0.001**
No	34 (63.0)	11 (32.4)	23 (67.6)		-
Fecalith detected					
Yes	7 (13.0)	100 (100.0)	0 (0.0)	-	0.013
No	47 (87.0)	23 (48.9)	24 (51.1)	-	-
Fat stranding present					
Yes	21 (38.9)	17 (81.0)	4 (19.0)	6.54 (1.79-22.84)	**0.005**
No	33 (61.1)	13 (39.4)	20 (60.6)		-
Free fluid detected					
Yes	25 (46.3)	19 (76.0)	6 (24.0)	5.18 (1.58-16.95)	**0.007**
No	29 (53.7)	11 (37.9)	18 (62.1)	-	-
Evidence of acute appendicitis					
Yes	25 (46.3)	25 (100,0)	0 (0,0)	-	**<0.001**
No	29 (53.7)	5 (17.2)	24 (82.8)	-	-
Evidence of perforated appendicitis					
Yes	7 (13.0)	7 (100)	0 (0.0)	-	0.731
No				-	-

Table 4: Use and characteristics of imaging.

Clinical Scores	**Cutoff**	**Accuracy**	**Sensitivity**	**Specificity**	**pos. DLR**	**neg. DLR**	**p value**
Alvarado score	> 6	0.681	0.608	0.767	2.609	0.511	**<0.001**
Laboratory values							
White blood cell count (WBC)	> 10	0.655	0.65	0.667	1.949	0.525	**<0.001**
C-reactive protein (CRP)	> 5	0.659	0.807	0.437	1.435	0.44	**<0.001**
Pancreatic stone protein (PSP)	< 40	0.607	0.897	0.256	1.206	0.402	**<0.001**
Imaging							
Ultrasound	Appendicitis	0.767	0.891	0.678	6.212	0.157	**<0.001**
Computer tomography	Appendicitis	0.907	0.833	1.000	-	0.167	**<0.001**

Table 5: Diagnostic accuracy analysis of test cut-offs for appendicitis.

What is the value of PSP in the differential diagnosis of right iliac fossa pain?

As mentioned above, PSP was higher in the patient group without appendicitis and any other condition causing right iliac fossa pain. In the 61 patients with a PSP value of >40 ng/ml, 20 (33%) were diagnosed with appendicitis while in the 293 patients with PSP <40, 174 (59%) had appendicitis (p<0.001) (Table 3, Figure 4). Thus, a PSP value higher than 40 ng/ml, indicates a lower likelihood for appendicitis in patient with right iliac fossa pain.

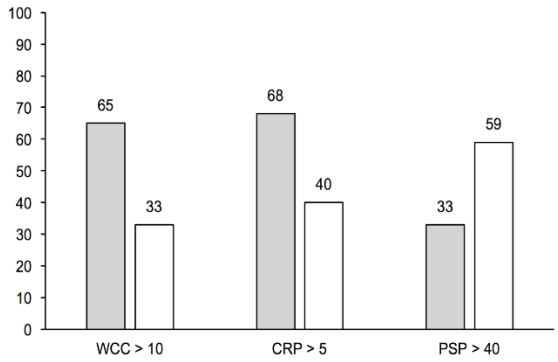

Figure 4: Laboratory values above and below the cut-off point for appendicitis (gray = appendicitis, white = no appendicitis / any other condition).

What is the value of the Alvarado score, laboratory values and imaging in predicting appendicitis on histology of patients receiving an operation?

	Patients with appendectomy (n=219)	Appendicitis (n=194)	No Appendicitis (n=25)	OR (95% CI)	Accuracy	Sensitivity	Specificity	p value
Alvarado score, median (i.q.r.)	7 (6-8)	7 (6-8)	6 (5-1)	-	-	-	-	**0.024**
Alvarado score grouped								-
Alvarado score 7-10	129 (59)	118 (61)	11 (44)	2.0 (0.9-4.6)	0.602	0.608	0.560	0.132
Alvarado score 0.6	90 (41)	76 (39)	14 (56)	-	-	-	-	-
White blood cell count (WBC)								
WBC, mean (SD)	13.2 (4,3)	13 (4)	12 (3)	-	-	-	-	0.784
WBC, median (i.q.r)	13 (11.15)	13 (11.16)	12 (9-15)	-	-	-	-	0.121
WBC> 10 grouped	132 (66)	119 (68)	13 (54)	1.8 (0.8-4.2)	0.744	0.793	0.360	0.25
WBC≤ 10 grouped	68 (64)	57 (32)	16 (46)	-	-	-	-	-
Missing WCC, n	19	-	-	-	-	-	-	-
C-reactive protein (CRP)								
CRP, mean (SD)	48 (55)	47 (52)	47 (59)	-	-	-	-	0.595
CRP, median (i.q.r)	31(6.61)	31(8.66)	30 (5-61)	-	-	-	-	0.625
CRP>5 grouped	173 (79)	155 (81)	18 (72)	1.6 (0.6-4.2)	0.747	0.896	0.159	0.299
CRP ≤ 5 grouped	44 (20)	37 (19)	7 (28)	-	-	-	-	-
Missing CRP, n	2	-		-	-	-	-	-
Pancreatic stone protein (PSP)								
PSP, mean (SD)	34 (68)	25 (33)	87 (165)	-	-	-	-	
POP, median (i.q.r)	18 (13-28)	18 (13-27)	21(13-59)	-	-	-	-	0.186
POP > 40 grouped	30 (14)	20 (10)	10 (40)	5.73 (20-15.9)	0.840	0.897	0.400	

	Patients with appendectomy (n=219)	Appendicitis (n=194)	No Appendicitis (n=25)	OR (95% CI)	Accuracy	Sensitivity	Specificity	p value
PSP ≤ 40 grouped	189 (86)	174 (90)	15 (60)	-	-	-	-	-
Missing POP, n	0	-	-	-	-	-	-	-
Acute appendicitis on ultrasound								
Yes	111 (85)	103 (68)	8 (40)	3.2 (1.2-8.2)	0.669	0.678	0.600	0.023
No	61 (35)	49 (32)	12 (60)	-	-	-	-	-
Acute appendicitis on CT								
Yes	25 (83)	25 (83)	0 (0.0)	-	0.833	1.000	-	-
No	5 (17)	5 (17)	0 (0.0)	-	-	-	-	-

Table 6: Predictors of appendicitis on histology.

In an attempt to improve the Alvarado likelihood score for acute appendicitis, PSP was included into the score by simply adding an additional point when PSP was less than 40 ng/ml. In 76% of the patients with appendicitis, the Alvarado score was more than 6. When PSP was added to the score, 85% of patients with appendicitis, demonstrated a positive score (p<0.001) (Figure 5). Thus, when PSP is more than 40 ng/ml, the diagnosis of appendicitis is less likely and further investigations, such as computer tomography, would be needed before the decision to operate.

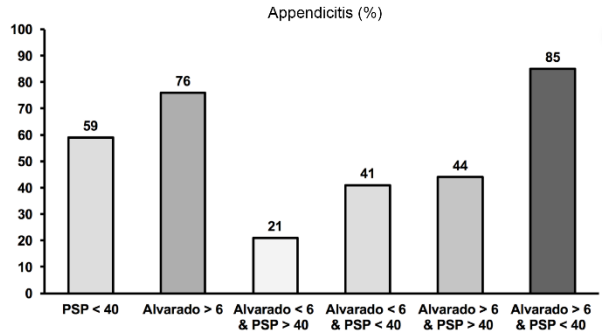

Figure 5: Integration of PSP in the Alvarado score for appendicitis.

The only laboratory value obtained at the emergency room that could discriminate appendicitis from a normal appendix on histology was PSP, with 92% (174/189) of patients with a PSP <40 ng/ml having appendicitis and only 8% (15/189 PSP<40) of patients with a PSP >40 ng/ml having appendicitis (p<0.001) (Figure 6).

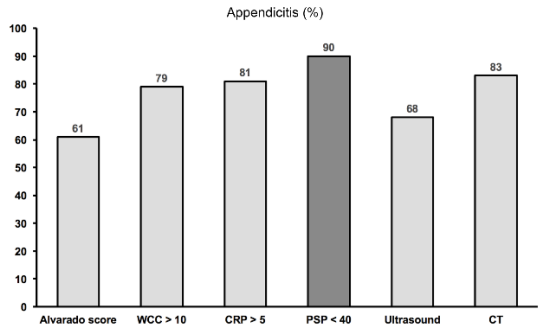

Figure 6: The value of the different tests in predicting appendicitis on histology.

Of the 30 patients who underwent CT and an appendectomy, 25 (83%) were correctly predicted with appendicitis while in 5 patients (17%), CT missed appendicitis (Table 6). Of all tests, PSP <40 ng/ml had the highest accuracy (84%, p<0.001) in predicting appendicitis on histology when compared to the Alvarado score>6 (60%, p=0.132), WBC > 10 x 10^9/l (74%, p=0.122), CRP >5 mg/l (75%, p=0.299), ultrasound (67%, p=0.023) and CT (83% p=n/a) (Table 6).

Discussion

The present study found that in patients with a suspicion of appendicitis, higher PSP levels are associated with less likelihood of having the suspected clinical entity. Inclusion of PSP levels in the Alvarado score seem to further improve its known predictive value. With a cut-off level of 40 ng/ml (when <40/positive), PSP

had the highest accuracy of 84% in predicting appendicitis on histology when compared to the Alvarado score, CRP, USS and CT. PSP were first discovered in patients with chronic pancreatitis [14] and is transiently elevated in patients with pancreatitis [15]. The protein is localized to the acinar cells of the pancreas [16]. As an acute-phase protein regulated by IL-6 and other cytokines, PSP is released after tissue injury. Studies in animal models revealed, that PSP is released upon stress even in the absence of chronic or acute pancreatitis. To a lower extent, PSP is also synthetized in the Paneth cells of the small intestine [17] and the fundus of the stomach while it is not produced in the colon and the appendix [18,19]. The fact that PSP is neither produced in the colon nor the appendix may explain why PSP is elevated in conditions such as gastroenteritis or gastritis but not elevated during appendicitis. In fact, PSP best excluded the diagnosis of appendicitis and therefore may reduce the negative appendectomy rate if integrated in the routine work-up.

WBC is useful in predicting appendicitis combined with the history and the clinical presentation [20]. The acute phase protein CRP increases with some delay and is a predictor for complicated appendicitis. However, studies have reported normal WBC levels and normal CRP levels in up to 10% of patient with histologically proven appendicitis [21]. Since normal inflammatory markers cannot exclude appendicitis, it still remains a primarily clinical diagnosis. This is taken into consideration in the Alvarado score. The Alvarado score was implemented in 1986 aiming for early diagnosis in patients with suspicion for appendicitis. It was modified in order to reduce the rate of negative appendectomy [22]. The Alvarado score includes laboratory findings, symptoms such as nausea, pain localization and anorexia in the scoring system. In addition, clinical signs like elevated body temperature and tenderness are represented. Modifications were suggested, however, according to a recently published systematic review, the original Alvarado score outperformed the modified Alvarado score across all three criteria [23]. USS is the most commonly used modality confirming the diagnosis of appendicitis after clinical suspicion with a sensitivity and specificity between 71% and 97% [24]. The limitations include examiner and patient dependent validity. CT has reduced the negative appendectomy rate to less than 10% (compared to 21% in the pre-CT era) however involves ionizing radiation and relatively high costs [25]. Diagnostic laparoscopy is an accurate diagnostic and treatment option when patient history, clinical examination and diagnostic tests are highly suspicious for an acute appendicitis, especially in young, female and otherwise healthy patients.

The current study has several limitations, including a potential selection and recruitment bias as well as statistical uncertainty. Patients with very early and self-limited appendicitis could have been missed as well as some patients discharged were lost to follow up. The subgroup analysis was performed in patients that were brought to surgery alone. The sample size calculation was conducted based on the test variable PSP and the primary outcome appendicitis on histology. The authors did not perform multiple sample size calculations addressing the remaining diagnostic tests. The fact that not all patients received imaging may results in underpowered calculations and comparisons. Furthermore, diagnostic modalities and decision to operate may have varied between centers. Another limitation involves the freedom of the investigators to perform or not imaging studies according to their clinical discretion. However, this study was a "pragmatic trial" allowing the use of imaging modalities only when indicated and not routinely as part of the study protocol. Another potential limitation is the fact that this multicenter study included 7 hospitals over an overall three-and-a-half-year period, with 357 patients recruited with suspicion of appendicitis. One may consider this number being low, however the recruitment starts and end date for each hospital differed significantly, mainly due to logistic, delay in ethics approval, and other delays. However, the authors do not consider this as an important limitation, as during the trial period, the diagnosis and management of acute appendicitis did not change over time. In addition, some data sets were incomplete.

The use of the PSP value in the diagnostic workup for appendicitis and in patients with abdominal pain may provide additional information to the doctors such as when to perform a CT, when to operate and when the complaint is highly suspicious for an enteritis or a gastroenteritis. There is a need for a prospective trial where PSP will be routinely included in the work up of appendicitis and the doctors are provided with the test result immediately. Furthermore, PSP should become a laboratory test for daily routine and as such available to the doctor shortly after ordering.

Conclusion

Higher PSP levels are associated with a lower likelihood of having acute appendicitis. With a cut off of 40 ng/ml (when <40/ positive), PSP had the highest accuracy in predicting appendicitis on histology when compared to the Alvarado score, CRP, USS and CT.

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