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Clinical, Molecular Biological, and Microbiological Integrated Investigation in the Case of Paediatric Acute Complicated and Uncomplicated Appendicitis

Summary of the Doctoral Thesis for obtaining the scientific degree “Doctor of Science (*PhD*)”

Sector Group – Medical and Health Sciences
Sector – Clinical Medicine
Sub-Sector – Surgery (Paediatric Surgery)

Riga, 2023

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Abbreviations

AA	Acute appendicitis
AcA	Acute complicated appendicitis
AuA	Acute uncomplicated appendicitis
AUC	Area under the receiver operating characteristic curve
CRP	C-reactive protein
Ctr	Control group
ESBL	Extended spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
IL-6	Interleukin-6
IQR	Interquartile ranges
LRG1	Leucine-rich alpha-2 glycoprotein 1
Mdn	Median
MLE	Maximum likelihood estimation
NGAL	Neutrophil gelatinase-associated lipocalin
ROC	Receiver operating characteristic
S-IL-6	Serum interleukin-6
S-LRG1	Serum leucine-rich alpha-2 glycoprotein 1
S-NGAL	Serum neutrophil gelatinase-associated lipocalin
U-IL-6	Urine interleukin-6
U-LRG1	Urine leucine-rich alpha-2 glycoprotein 1
U-NGAL	Urine neutrophil gelatinase-associated lipocalin
US	Ultrasound

Introduction

Aim

To evaluate new urine and serum biomarkers, bacterial aetiology and antibacterial susceptibility for the early and accurate diagnosis of acute appendicitis (AA), and differentiation of acute uncomplicated (AuA) and acute complicated (AcA) appendicitis in paediatric patients.

Objectives

The following objectives are set to reach the aim of the investigations:

1. Determine serum levels of inflammatory biomarkers (CRP), interleukin-6 (IL-6), neutrophil gelatinase-associated lipocalin (NGAL), and leucine-containing alpha glycoprotein 1 (LRG1) in patients with a diagnosis of AA.
2. Determine the level of inflammatory biomarkers (CRP, IL-6, NGAL and LRG1) in the urine of patients with a diagnosis of AA.
3. Determine the serum level of *Yersinia enterocolitica* antibodies in patients with a diagnosis of AA.
4. Identify the causative agents of AuA and AcA, to evaluate their antibacterial sensitivity.

Hypothesis

- The role of blood serum biomarkers NGAL and LRG1 and urinary biomarker LRG1 is essential in the early diagnosis of AcA and differentiate AuA from AcA in children aged seven to 18 years.
- Appendiceal microbiota and antibacterial susceptibility of causative agents may contribute to the treatment of acute complicated and uncomplicated appendicitis in paediatric patients.

Novelty

The study shows that the urine biomarker LRG1 plays an important diagnostic and differentiating role in the uncomplicated and complicated form of AA. Urine can be obtained non-invasively. The U-LRG1 detection method provides a quick result and gives an opportunity to evaluate future treatment tactics.

The study has proved the antibacterial sensitivity of the most common AcA bacteria, which will allow to develop an algorithm of antibacterial therapy in cases of AuA and AcA

1 Materials and methods

1.1 Study setting and study population

The research was arranged as a prospective, single-centre, controlled-group study including children between the ages of 7 and 18 admitted to the Children's Clinical University Hospital due to acute abdominal pain with signs and symptoms suggesting the possibility of appendicitis. All patients were examined to confirm or exclude this diagnosis. Before inclusion in the study, physical condition examination, complete blood count, abdominal US, and determination of serum IL-6 values were performed.

All procedures performed in this study involving patients were conducted in accordance with the ethical standards of the institutional and/or national research committee, and the studies comply with the requirements of the Patient's Data Protection Law and the principles of the Declaration of Helsinki. The approval of the Ethics Committee's was received by both the Children's Clinical University Hospital and Riga Stradiņš University (reference number: SP-37/2018 and 21/27.04.2017, respectively) for the period from January 2017 to 2020, during which the research was conducted. Clinical data collected preoperatively included patients' age, sex, and current medical history.

The study group included patients with confirmed AA and were treated with an appendectomy, either laparoscopic or conventional laparotomy. Patients suspected of having appendicitis, but having previous abdominal surgery, pregnancy, and chronic medical conditions that could potentially affect the renal, gastrointestinal, or respiratory systems were excluded. The control group (Ctr) included patients without any suspected inflammatory process in the respiratory, renal, or gastrointestinal tracts, but were admitted to the emergency department, mainly with mild traumatic lesions.

Microbiological culture swabs from the appendix and abdominal cavity were collected intraoperatively. Depending on the intraoperative and bacteriological findings, two groups were established – AcA and AuA.

Researchers handed a written consent form to the caregiver and to the patient if they were 13 years or older. The consent form provided the patient and caregiver with information about the purpose of the study and the methodology for investigating biological material. Informed consent was received from the parent of each participant in the study.

1.2 Statistical analysis

Microsoft Excel 2016 and (Microsoft, USA) IBM SPSS Statistics 27 (IMB, USA) were used for statistical analyses, and all data was validated by a certified statistical analyst to ensure accuracy. The median values and interquartile ranges (IQR) were used to express the results for quantitative data. The comparisons between groups were calculated using the Mann-Whitney U-test for two groups and the Kruskal-Wallis test for all three groups of quantitative variables, for non-parametric distribution. Pearson Chi-square and Fisher Exact Tests were applied on nominal variables to determine associations between them.

In the study on diagnostic biomarkers for AA, a receiver operated characteristic (ROC) curve was generated by plotting the false-positive fraction versus the true-positive fraction for every possible cut-off score, and area under the ROC curve (AUC) was calculated, this determined the clinical importance of the biomarkers, as well as their diagnostic value regarding appendicitis.

Binary logistic regression was used as an appropriate statistical technique when the dependent variable is binary. It represents two groups of interest with values of 0 and 1, such as yes/no, presence/absence or success/failure. The procedure for estimating coefficients is maximum likelihood, and the goal is to

find the best linear combination of independent variables to maximise the likelihood of obtaining the observed outcome frequencies. The predictive values of biomarkers were evaluated by receiver operating characteristics curve (ROC) and binary logistic regression models. Two different models were analysed – AA vs Ctr and AcA vs AuA. A p value of < 0.05 was associated with statistical significance.

2 Results

2.1 Results – biomarkers

2.1.1 Demographics characteristics of the patients

Samples were collected from 153 patients eligible for this research. 97 (63.4 %) (AuA or AcA) were diagnosed with appendicitis and 56 (36.6 %) had no suspected infectious or inflammatory pathology (Ctr). Participant age ranged from seven to 18 years, with a median of 13 (IQR 10.0–15.0) years (AuA), 12 (IQR 9.0–14.0) years (AcA) and 13.5 (IQR 10.3–15.0) years (Ctr). 89 (58.2 %) of them identified as boys and 64 (41.8 %) girls.

Suspicion of appendicitis that required an urgent diagnostic laparoscopy in 85 (87.6 %) patients and laparotomy in 12 (12.4 %) of the cases (there were four AuA patients and eight AcA patients). Intraoperative swabs of free peritoneal liquid were collected. Patients with positive culture from samples of the peritoneal cavity were classified in the AcA group, with 52 patients (53.6 %), and those with a negative culture were classified in the AuA group – 45 patients (46.4 %).

Nine AuA (22.5 %) and 31 AcA (77.5 %) patients required the placement of a drainage tube. More than half of the patients (60.8 %) that had a drainage tube inserted were diagnosed with AcA ($p < 0.001$). A simple comparison suggests that AcA patients had a slightly longer median postoperative hospital stay, six versus five days for AuA patients.

2.1.2 Preoperative and postoperative biomarker levels

Baseline preoperative values of IL-6, NGAL and LRG1 are presented in Table 2.1, along with the values of the second and the fifth postoperative days. The lowest baseline level (at the start of the study or Day 0) of all observed parameters was found in the control group (Ctr) without infectious disease, whilst

the highest was observed in AcA. The drastic decrease in levels of biomarkers S-IL-6 and S-NGAL can be observed from Day 0 until Day 5, as the inflammation settles postoperatively; data is presented in Table 2.1.

Table 2.1

Preoperative and postoperative biomarker levels

Biomarkers		**AuA, ng or pg or µg/ml (IQR)**	**AcA, ng or pg or µg/ml (IQR)**	**Ctr, ng or pg or µg/ml (IQR)**	**p value**
Day 0					
Serum	IL-6	22.57 (11.15–42.21)	70.59 (25.06–300.92)	6.44 (2.49–12.49)	< 0.001
	NGAL	128.20 (81.44–184.50)	169.90 (104.95–258.15)	90.37 (73.46–137.38)	< 0.001
	LRG1	70.56 (62.64–83.43)	88.12 (71.12–106.13)	34.08 (27.50–42.37)	< 0.001
Urine	IL-6	2.37 (0.55–27.93)	11.22 (2.82–29.10)	6.84 (1.37–38.98)	0.227
	NGAL	2.93 (1.41–8.57)	3.34 (1.10–10.45)	3.25 (1.41–10.73)	0.889
	LRG1	0.10 (0.03–0.73)	0.35 (0.05–1.38)	0.04 (0.02–0.10)	< 0.001
Day 2					
Serum	IL-6	7.48 (2.81–23.44)	13.55 (6.84–33.73)	–	0.131
	NGAL	73.67 (58.04–92.41)	107.10 (71.04–167.20)	–	0.001
	LRG1	74.99 (61.00–96.03)	87.90 (70.32–104.10)	–	0.048
Urine	IL-6	4.42 (1.15–16.97)	6.89 (2.04–21.71)	–	0.439
	NGAL	2.66 (1.34–12.18)	2.65 (0.81–9.87)	–	0.633
	LRG1	0.08 (0.03–0.28)	0.21 (0.06–0.98)	–	0.017

Table 2.1 continued

Biomarkers		AuA, ng or pg or µg/ml (IQR)	AcA, ng or pg or µg/ml (IQR)	Ctr, ng or pg or µg/ml (IQR)	p value
Day 5					
Serum	IL-6	4.45 (2.40–10.70)	5.07 (1.72–12.48)	–	0.838
	NGAL	69.80 (60.20–89.99)	85.25 (64.20–105.50)	–	0.220
	LRG1	66.73 (56.98–85.28)	80.97 (62.14–99.03)	–	0.110
Urine	IL-6	2.44 (0.65–8.56)	2.18 (0.00–10.82)	–	0.900
	NGAL	4.89 (1.26–13.50)	2.39 (1.17–5.46)	–	0.281
	LRG1	0.04 (0.02–0.27)	0.10 (0.03–0.25)	–	0.102

Biomarker levels are expressed as medians, IQR (25 %, 75 %), IL-6 is measured in pg/ml, NGAL in ng/ml and LRG1 in µg/ml, AcA – acute complicated appendicitis, AuA – acute uncomplicated appendicitis, Ctr – Control, IL-6 – Interleukin-6, NGAL – Neutrophil Gelatinase-associated Lipocalin, LRG1 – Leucine-rich Alpha-2 Glycoprotein 1. #The group did not undergo abdominal surgery; thus, only biomarkers of Day 0 are included in this study

2.1.3 Serum IL-6 levels

The median S-IL-6 Day 0 levels for AuA, AcA and Ctr group were 22.57 pg/ml, 70.59 pg/ml, and 6.44 pg/ml respectively. The distribution of S-IL-6 on Day 0 was statistically higher (Kruskal-Wallis test, T stat = 63.32, $p < 0.001$) in AcA compared to the control group (Pairwise comparison, $p < 0.001$), as well as the distribution of S-IL-6 was statistically higher in AcA compared with AuA (Pairwise comparison, $p = 0.007$) and in AuA compared with the control group (Pairwise comparison, $p < 0.001$). A decrease in the levels of biomarkers can be observed between Day 0 and Day 5, as the inflammation settles postoperatively.

2.1.4 Urine IL-6 levels

U-IL-6 samples were inconclusive and, thus, not specific enough to differentiate between AcA and AuA or AA from the Ctr group.

2.1.5 Serum NGAL levels

The median S-NGAL Day 0 levels for AuA, AcA and the Ctr were 128.20 ng/ml, 169.90 ng/ml, and 90.37 ng/ml respectively. The distribution of S-NGAL on Day 0 was significantly different (Kruskal-Wallis tests, T stat = 19.04, $p < 0.001$) in AcA compared with the Ctr (Pairwise comparison, $p < 0.001$). S-NGAL values of AuA were higher than those of the Ctr (Pairwise comparison, $p = 0.087$). Thus, the S-NGAL values in AcA were the highest. S-NGAL levels on the second postoperative day decreased to 107.1 ng/ml in AcA and 73.67 ng/ml in AuA ($p = 0.001$). S-NGAL levels on the fifth postoperative day decreased to 85.25 ng/ml in AcA and 69.8 ng/ml in AuA ($p = 0.220$).

2.1.6 Urine NGAL levels

The U-NGAL samples were inconclusive and thus not specific enough to differentiate between AcA and AuA or AA from Ctr group.

2.1.7 Serum LRG1 levels

The median S-LRG1 on Day 0 levels for AuA, AcA and Ctr were 70.56 μg/ml, 88.12 μg/ml, and 34.08 μg/ml respectively. The distribution of serum LRG1 on Day 0 was significantly different (Kruskal-Wallis test, T stat = 88.30, $p < 0.001$) in AcA compared with the Ctr (Pairwise comparison, $p < 0.001$); equally, the distribution of S-LRG1 was significantly different in AuA compared with Ctr (Pairwise comparison, $p < 0.001$). LRG1 values of AcA

were higher than those of AuA (Pairwise comparison, $p = 0.074$). S-LRG1 levels declined to 80.97 μg/ml and 66.73 μg/ml in AcA and AuA ($p = 0.110$) respectively on the fifth postoperative day, which were also significantly lower than levels at Emergency Department (ED) admission ($p < 0.001$).

Additional assessment of the dependency between S-LRG1 concentration and disease grade in AA patients revealed that appendiceal mucosal inflammation significantly correlates with an increased S-LRG1. There was a significant difference between control and AcA and/or AuA ($p < 0.001$, $p < 0.001$), as well as disease severity $p = 0.001$ when compared between AcA versus AuA only.

2.1.8 Urine LRG1 levels

The urine sample of the LRG1 Day 0 level biomarker was conclusive, thus denoting a significant difference between AcA and the Ctr as well as between AuA and the Ctr. Day 0 values are 0.35 μg/ml (AcA), 0.1 μg/ml (AuA) and 0.04 μg/ml (Ctr). There was a significant difference between the Ctr versus AcA and AuA ($p < 0.001$, $p = 0.005$).

U-LRG1 levels on the fifth postoperative day decreased to 0.10μg/mL in AcA and 0.04μg/mL AuA ($p = 0.102$). Urine LRG1 levels were significantly higher at the time of admission to the ED than on the fifth postoperative day ($p < 0.001$).

Further assessment of whether U-LRG1 levels were associated with disease activity in patients with AA revealed that appendiceal mucosal inflammation significantly correlates with increased U-LRG1 levels ($p = 0.001$). There was a significant difference between control versus AcA and AuA ($p < 0.001$, $p = 0.005$), however, disease severity (AcA vs. AuA) could not be differentiated ($p = 0.089$).

U-LRG1 levels on the fifth postoperative day declined to 0.10 μg/ml in AcA and 0.04 μg/ml AuA ($p = 0.102$). U-LRG1 levels were significantly higher at the time of admission to the ED than on the fifth postoperative day, ($p < 0.001$).

2.1.9 Comparison between serum and urine biomarker levels

The urine samples for all three biomarkers were collectively inconclusive, and thus not specific enough to differentiate between AcA and AuA. If we compare AcA with AuA, a significant difference between baseline (Day 0) S-IL-6, S-NGAL and S-LRG1 individually ($p < 0.001$, $p = 0.033$, and $p = 0.001$) could be seen.

2.1.10 Threshold sensitivity and specificity of biomarkers

The S-IL-6 cut-off value in patients with AA was 20.25 pg/ml; S-NGAL cut-off was 103.75 ng/ml and S-LRG1 cut-off was 51.69 μg/ml ($p < 0.001$).

The ROC curves demonstrated AUC of 0.856 (95 % CI 0.798–0.915), AUC of 0.689 (95 % CI 0.604–0.773) and AUC of 0.945 (95 % CI 0.905–0.985) respectively. The ROC curve for U-LRG1 demonstrated AUC of 0.703 (95 % CI 0.619–0.787) and CRP AUC of 0.851 (95 % CI 0.790–0.931). IL-6 for appendicitis had a sensitivity of 71.9 % and specificity of 91.1 %, while S-LRG1 showed a higher sensitivity and specificity of 93.8 % and 91.1 % respectively.

The binary logistic regression shows that among the biomarkers taken on admission IL-6 and LRG1 were significantly associated with appendicitis diagnostic. The binary logistic regression model was estimated using the maximum likelihood estimation (MLE) procedure. The overall model 1 was statistically significant: model X^2 (3. 153) = 126.446 with a p value of < 0.001. The Hosmer-Lemeshow test was used to evaluate the goodness-of-fit of the model. The resulting test statistic was not statistically significant ($X^2 = 5.518$,

p = 0.701), therefore the null hypothesis (H0: there is no difference between the observed and the model predicted values of the appendicitis) was rejected. This implied that the model fit the data well at a statistically acceptable level. Consequently, the model was able to predict correctly 92.8 % of those who have an appendicitis (1) and 89.3 % of those who have a do not have appendicitis (0). Overall, 91.5 % of all cases (0.1) were correctly predicted. Another test statistic, the Nagelkerke R^2, was used to measure the usefulness of the model which indicates how useful the explanatory variables were in predicting the response variable. The Nagelkerke R^2, varies from 0 and 1, was 0.769 indicating the model was useful in predicting appendicitis. The Wald and associated p value are used to test the statistical significance of each coefficient (B) in the model.

Also, overall model 2 was statistically significant: model 2 (3.97) = 17.070 with a p value of 0.001. The Hosmer-Lemeshow test statistic was not statistically significant ($X^2 = 14.696$, p = 0.065) which implied that the model fit the data well at a statistically acceptable level. Consequently, the model was able to predict correctly 65.4 % of those who have complicated appendicitis (1) and 77.8 % of those who have a do have uncomplicated appendicitis (0). Overall, 71.1 % of all cases (0.1) were correctly predicted. The Nagelkerke R^2 was 0.216 indicating the model was useful in predicting complicated appendicitis.

The combined diagnostic model of IL-6, LRG1, NGAL in serum was established by binary logistic regression analysis. The ROC curve showed that combined diagnostic model 1 (AA vs Ctr) reached a sensitivity of 92.8 %, a specificity of 89.3 % and an area under the curve of 0.96 (95 % CI 0.93–0.99, p < 0.001). The ROC curve showed that combined diagnostic model 2 (AcA vs AuA) reached a sensitivity of 67.3 %, a specificity of 77.8 % and an area under the curve of 0.74 (95 % CI 0.63–0.84, p < 0.001).

2.2 Results – microbiota and antibacterial susceptibility

Escherichia coli was the prevalent representative of appendiceal intraluminal microbiota in both complicated and uncomplicated cases, totalling 79 patients (81.4 %). *Pseudomonas aeruginosa* was the prevalent microorganism of the extraluminal appendiceal microbiota (AcA/AuA: 15/5). There were some differences in the microbiota of the proximal and distal parts of the appendix between patients with acute complicated and acute uncomplicated appendicitis. In the AcA group, 35 cases (55 %) had identical microbiota, while in the remaining 17 cases (35 %) the microbiota differed in distal and proximal parts. In the AuA group, 24 (53 %) cases had identical microbiota, but in 21 cases (47 %) they differed.

Yersinia enterocolitica antibody detection preoperatively was negative in all cases.

Bacterial culture resulted in positive intraluminal samples with the growth of one or several isolates from each appendix. Table 2.2 shows the number of cases of the most common isolates per the subdivision AcA and AuA. Frequently, mixed strains were found at culture. The most common bacteria isolated from the appendix were *Escherichia coli* in 79, followed by *Pseudomonas aeruginosa* in 20, *Klebsiella pneumoniae* in 6, *Bacterioides fragilis* in 5, and *Citrobacter braakii* in five samples.

Table 2.2

Types of isolated bacteria, frequency, and percentage in both acute complicated appendicitis and acute uncomplicated appendicitis

Indicator	AcA		AuA		Total Isolates, No.	p value
	No.	%	No.	%		
Escherichia coli	43	54.4	36	45.6	79	0.424#
Pseudomonas aeruginosa	15	75	5	25	20	0.024#
Klebsiella pneumoniae	2	33.3	4	66.7	6	0.417#
Citrobacter braakii	0	0	3	100	3	0.102*

Table 2.2 continued

Indicator	AcA		AuA		Total Isolates, No.	p value
	No.	%	No.	%		
Bacterioides fragilis	2	40	3	60	5	0.665*
Kocuria kristinea	1	50	1	50	2	>0.999*
Other cases	40	64.5	22	35.5	62	0.001#
Total	103	–	74	–	177	–

AcA – acute complicated appendicitis; AuA – acute uncomplicated appendicitis; Other cases – other or/and mixed from others; # – Pearson Chi-square test; * – Fisher Exact test.

The 79 samples isolating *E. coli* had various antibacterial sensitivities such as five strains (8.5 %) were resistant to ceftazidime; 32 (54.2 %) to ampicillin; six (10.2 %) to cefotaxime; six (10.2 %) to imipenem; eight (13.6 %) to ciprofloxacin; six (10.2 %) to chloramphenicol; two (3.4 %) to ertapenem; 18 (30.5 %) to amoxicillin/clavulanic acid, one (1.7 %) to piperacillin-tazobactam, and one (1.7 %) to gentamicin. All strains were susceptible to meropenem and amikacin. Additionally, five ESBL-producing strains of *E. coli* were also isolated.

P. aeruginosa, the second most common causative agent, showed a high prevalence in acute complicated appendicitis cases. A good response was shown during susceptibility testing to ceftazidime with only 26.3 % of isolates being resistant. Ampicillin resistance was noted in 78.9 % of isolates, while in 63.2 % to cefotaxime, in 36.8 % to imipenem, in 52.6 % to chloramphenicol, in 10.5 % to ciprofloxacin and piperacillin/tazobactam, in 63.2 % to ertapenem and in 84.2 % to amoxicillin/clavulanic acid. All tested strains were susceptible to meropenem, amikacin and gentamicin. Antibacterial susceptibility of other bacteria that were isolated in this study are shown in Table 2.3. *Citrobacter spp*. tested resistant to all antibiotics except for amoxicillin/clavulanic acid, while *Klebsiella spp.* was resistant to cefotaxime, amikacin, gentamicin as well as chloramphenicol.

Table 2.3

Antimicrobial resistance and susceptibility of isolated pathogens

Indicator	*E. coli* n, %		*P. aeruginosa* n, %		*Klebsiella* n, %		*Citrobacter* n, %	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**
CAZ	5	54	5	14	1	8	–	5
	8.5	91.5	26.3	73.7	11.1	88.9	–	100
AMP	32	27	15	4	7	2	–	5
	54.2	45.8	78.9	21.1	77.8	22.2	–	100
CTX	6	53	12	7	–	9	–	5
	10.2	89.8	63.2	36.8	–	100	–	100
MRP	–	59	–	19	1	8	–	5
	–	100	–	100	11.1	88.9	–	100
IMI	6	53	7	12	1	8	–	5
	10.2	89.8	36.8	63.2	11.1	88.9	–	100
AK	–	59	–	19	–	9	–	5
	–	100	–	100	–	100	–	100
CN	1	58	–	19	–	9	–	5
	1.7	98.3	–	100	–	100	–	100
CIP	8	51	2	17	1	8	–	5
	13.6	86.4	10.5	89.5	11.1	88.9	–	100
C	6	53	10	9	–	9	–	5
	10.2	89.8	52.6	47.4	–	100	–	100
ETP	2	57	12	7	1	8	–	5
	3.4	96.6	63.2	36.8	11.1	88.9	–	100
AUG	18	41	16	3	2	7	5	–
	30.5	69.5	84.2	15.8	22.2	77.8	100	–
TZP	1	58	2	17	1	8	–	5
	1.7	98.3	10.5	89.5	11.1	88.9	–	100

Abbreviations: CAZ – ceftazidime, AMP – ampicillin, CTX – cefotaxime, MRP – meropenem, IMI – imipenem, AK – amikacin, CN – gentamicin, CIP – ciprofloxacin, C – chloramphenicol, ETP – ertapenem, AUG – amoxicillin/clavulanic acid, TZP – piperacillin/tazobactam.

Conclusions

1. Biomarker U-LRG1 is an accurate marker in AA diagnosis confirmation. Novelty is in its detection in the urine sample, therefore, is non-invasive and quick test. Concentration of serum and urine LRG1 is useful in detecting the severity of AA with respect to AcA and AuA.
2. Biomarker serum NGAL increases significantly at admission in the emergency department (Day 0) and should be used in the differential diagnosis of acute abdominal pain.
3. Although CRP and serum IL-6 remain as unspecific biomarkers but still can be used for diagnosis of AA and differentiation of AcA and AuA.
4. *P. aeruginosa* is identified more frequently in acute complicated appendicitis, and is susceptible to agents of the cephalosporin group, such as ceftazidime; however, *P. aeruginosa* has phenotypic resistance to cefotaxime. Therefore, cefotaxime should be removed from the empirical treatment algorithm of acute complicated appendicitis.
5. The incidence of ESBL-producing microorganisms was low in acute appendicitis cases included in the study.
6. Antibodies against *Yersinia enterocolitica* were not detected in the serum of AA patients, so they cannot be used as a prognostic criterion for AA.

Proposals

Considering the obtained results, it would be recommended to use serum and urine LRG1 (S-LRG1 and U-LRG1) and serum NGAL (S-NGAL) biomarkers in daily clinical practice in the diagnosis of acute appendicitis and in the differentiation of complicated and uncomplicated cases.

According to the obtained antibacterial sensitivity results, which were determined for the isolated microorganisms, it would be desirable to improve the antibacterial therapy guidelines for paediatric patients in the treatment for acute appendicitis, including ~~the~~ Children's Clinical University Hospital in Latvia.

Publications, reports, and patent on topics of doctoral thesis

Publications

1. Kakar, M., Delorme, M., Broks, R., Asare, L., Butnere, M., Reinis, A., Engelis, A., Kroica, J., Saxena, A., & Petersons, A. 2020. Determining acute complicated and uncomplicated appendicitis using serum and urine biomarkers: interleukin-6 and neutrophil gelatinase-associated lipocalin. Pediatric surgery international, 36(5), 629–636. https://doi.org/10.1007/s00383-020-04650-y

2. Kakar, M.; Berezovska, M.M.; Broks, R.; Asare, L.; Delorme, M.; Crouzen, E.; Zviedre, A.; Reinis, A.; Engelis, A.; Kroica, J.; Saxena, A.; Petersons, A. 2021. Serum and Urine Biomarker Leucine-Rich Alpha-2 Glycoprotein 1 Differentiates Pediatric Acute Complicated and Uncomplicated Appendicitis. Diagnostics 2021, 11, 860. https://doi.org/10.3390/diagnostics11050860

3. Kakar, M., Reinis, A., Kroiča, J., Eņģelis, A., Broks, R., Asare, L., Vermeulen, M., Senica, S. O., Saxena, A., & Pētersons, A. 2022. Microbiota Assessment of Pediatric Simple and Complex Acute Appendicitis. Medicina (Kaunas, Lithuania), 58(9), 1144. [1144]. https://doi.org/10.3390/medicina58091144

4. Kroiča, J., Reinis, A., Kakar, M., Delorme, M., Broks, R., Asare, L., Berezovska, M., Janšins, V., Zviedre, A., Enģelis, A., Saxena, A., & Pētersons, A. 2020. Culture Based Evaluation of Microbiota in Children with Acute Appendicitis. Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences., 74(2), 100–105. https://doi.org/10.2478/prolas-2020-0016

Patents registered at the Patent Board of Latvia

1. Kakar, M., Reinis, A., Broks, R., Eņģelis, A., Kroiča, J., Pētersons, A., Asare, L. 2022. Gangrenoza apendicīta attīstības riska noteikšanas paņēmiens bērniem (Patents Nr. 15613). Latvijas Republikas Patentu valde. http://databases.lrpv.gov.lv/databases/lv/patent/details/lvp2021000038/

Reports at international congresses and conferences

1. Complex clinical, biological and microbiological analysis of acute complicated and acute uncomplicated appendicitis in children. 2018. The 15th conference of the Baltic Association of Paediatric Surgeons. Riga, Latvia, May 10–12, 2018, 59.

2. Determining Acute Complicated and Uncomplicated Appendicitis Using Serum and Urine Biomarkers: Neutrophil Gelatinase-associated Lipocalin (NGAL), Leucine-rich Alpha-2 Glycoprotein 1 (LRG-1) and Interleukin6 (IL-6). 2019. The 6th World Congress of the World Federation of Associations of Pediatric Surgery (WOFAPS 2019). Abstracts. – Doha, Qatar, November, 1–3, 2019.

3. Determining acute complicated and uncomplicated appendicitis using serum and urine biomarkers: interleukin-6 and neutrophil gelatinase-associated lipocalin. 2020. 33rd International Symposium on Pediatric Surgical Research. Abstracts.- Frankfurt (Main), Germany, November, 27–29, 2020.

4. Evaluation of appendical microbiota in children with acute complicated and un complicated appendicitis. 31. 2020. Abstract from IX Latvian Gastroenterology Congress, Riga, Latvia. https://www.gastroenterologs.lv/content_graphics/user_file/pdf/Gastroenterologu_kongress_Abstracts_ENG_WEB.pdf

5. Biomarkers Leucine Rich Alpha-2 Glycoprotein 1, Neutrophil Gelatinase-Associated Lipocal and Interleukin-6 Diagnose Pediatric Acute Appendicitis. 2021. Poster session presented at 22nd Annual Congress of the European Paediatric Surgeons' Association (EUPSA), Athens, Greece.

6. Diagnostic Accuracy Of Multiple Biomarkers In Predicting The Severity Of Acute Pediatric Appendicitis In The Emergency Department. 2022. 23rd European Paediatric Surgeons' Association (EUPSA 2022) Congress. Abstract. Tel Aviv, Israel, June, July, 29–2.

7. Role of multiple biomarkers in predicting the diagnosis and severity of acute appendicitis in children. 214. 2022. Abstract from 7th World Congress of the World Federation of Associations of Pediatric Surgeons (WOFAPS), Prague, Czech Republic.https://guarant.eu/wofaps2022/login/files/wofaps-2022-book-of-abstracts.pdf

8. Microbiota diversity affects acute appendicitis severity in children. 2023. 16th Conference of the Baltic Association of Paediatric Surgeons. Abstract. Kaunas, Vilnius, September 14–16.

9. Predictive diagnostic biomarkers determine the severity of acute pediatric appendicitis: single center cohort study. 2023. 16th Conference of the Baltic Association of Paediatric Surgeons. Abstract. Kaunas, Vilnius, September 14–16.

10. Diagnostic precision of multiple biomarkers in predicting the outcome of acute appendicitis in pediatric patients. 2023. 36th International symposium on pediatric surgical research. Abstract. Milan, Italy, October 6-8.

11. Relationship of identified microbiota in severity of acute appendicitis in children. 2023. 36th International symposium on pediatric surgical research. Abstract. Milan, Italy, October 6-8.