doi:10.25143/prom-rsu_2023-23_dt

Mohit Kakar

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

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

Mohit Kakar

ORCID 0000-0001-5765-8207

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

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)

Supervisors of the Doctoral Thesis:

Dr. med., Professor **Arnis Enģelis**, Rīga Stradiņš University, Department of Paediatric Surgery and Children's Clinical University Hospital, Latvia

Dr. med., Professor **Juta Kroiča**, Chief of the Department of Biology and Microbiology, Rīga Stradiņš University, Latvia

Scientific Advisors:

Dr. habil. med., Professor **Amulya Saxena**, Chelsea Children's and Westminister Hospital, Imperial College London, United Kingdom

Dr. med., Assistant Professor **Aigars Reinis**, Department of Biology and Microbiology, Rīga Stradiņš University, Latvia

This research received grant support from the Latvian Council of Science and Rīga Stradiņš University

Riga, 2023

Abstract

Appendicitis poses a challenge throughout the entire process from diagnosis to effective treatment in paediatric patients. Considering that non-surgical or conservative treatment is used to treat acute appendicitis (AA) in children, this leads to one of the most important emergent problems in paediatric surgery – to differentiate between acute uncomplicated appendicitis (AuA) and acute complicated appendicitis (AcA) during the onset of treatment because AcA attests to the delayed diagnostic processes and requires only emergency surgical treatment. In Latvia, AcA occurs in more than 35 % of cases. Our previous studies have shown that at the Children's Clinical University Hospital (CCUH) Riga, Latvia, the total number of operated AA cases remains unchanged, however, the number of AcA cases is increasing.

The current diagnostic dilemmas of AcA show the need to search for new early diagnostic indicators in paediatric patients in order to reduce the incidence of complications and prevent lethal risks. The first challenge in the diagnosis of AA is the differentiation between AA and differential diagnoses that are non-appendicitis (nAA). Currently, the diagnosis of AA relies on typical clinical findings, anamnesis and the Alvarado score, all of which lack sensitivity and specificity and rely on the cooperation and fluency of patients and/or their carers. Other techniques such as computed tomography (CT) and diagnostic laparoscopy are used, but the negative aspects outweigh the productivity of the diagnostic value (Podany et al., 2017). The introduction of ultrasound (US) imaging and blood test values (leucocytosis and an increased C-reactive protein (CRP)) as diagnostic tools has decreased the need for diagnostic laparoscopies, but despite this, the current negative appendectomy rate is still at 1–40 % (Maloney et al., 2019). This research primarily aims to find a more productive solution to effectively diagnose AA by differentiating between AA and nAA.

It is already known that AcA is an inflammation of the complex origin of the appendix. Although it was traditionally considered to be simply an obstruction of the appendiceal lumen, there is increasing evidence that the disease can be caused by specific pathogenic microorganisms, with *Yersinia enterocolitica* being more specific (Fernandes et al., 2020). Some previous studies have confirmed a polymicrobial process, but it is not yet possible to identify the main pathogen and its source (Rogers et al., 2016); (Salö et al., 2017); (Bhattacharya et al., 2022); (Camacho-Cruz et al., 2022).

This has also led to increased interest and research in antibiotic therapy as a non-invasive treatment for AA. The increased use of antibiotics raises questions about the prevalence of microorganisms as causative agents in appendicitis, which is the focus of the present study. Differentiation between AuA and AcA is also an increasing problem, especially in emergency situations, and the prevalence of specific microorganisms for each of these classifications

essentially leads to a different antibiotic treatment strategy. Therefore, this study additionally aims to identify which microorganisms are more prevalent in AuA and AcA respectively as well as the subsequent proposed antibiotic strategy.

This also leads to the challenging task of rapid confirmation of AA and further differentiation between AuA and AcA in an emergency. Atypical differentiation and multiple differential diagnoses are two of many factors that hinder this rapid confirmation. Therefore, this study aims to further enable rapid diagnosis of AA and differentiation between AuA and AcA in an emergency setting.

The focus on immunological pathways is expanding and, consequently, the number of proposed biomarkers is increasing, although none has achieved widespread use to date (Selleck et al., 2017). The search for the optimal biomarker may be futile, but in combination with a medical history and clinical findings, it is possible to improve the quality of diagnostic approaches, thereby reducing complications and overall hospital costs incurred by reducing unnecessary imaging and surgery.

This was a prospective cohort study. The patients were divided into three groups: AcA (acute complicated appendicitis), AuA (acute uncomplicated appendicitis) and a Ctr (control group). Out of a total of 153 patients, 97 had AA (acute appendicitis) and 56 were in the Ctr. Our results show that urine leucine-rich alpha-2 glycoprotein 1 (LRG1) is an accurate marker in confirming the diagnosis of AA. The concentration of serum and urine LRG1 is useful in detecting the severity of AA with respect to AcA and AuA. The biomarker serum neutrophil gelatinase-associated lipocalin (NGAL) increases significantly on Day 0 and should be used in the differential diagnosis of acute abdominal pain. CRP and serum interleukin-6 (IL-6) remain non-specific biomarkers due to limitations and can be used to diagnose AA and differentiate AcA from AuA. *Pseudomonas aeruginosa* is more commonly identified 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 excluded from empirical treatment of acute complicated appendicitis. Antibiotic treatment strategies for acute complicated appendicitis should include antibiotics with different mechanisms of action to achieve a synergistic effect and prevent the development of antibiotic resistance. The incidence of extended-spectrum beta-lactamase (ESBL)-producing microorganisms was low in these cases of acute appendicitis. Serum antibodies to *Yersinia enterocolitica* were not detected in AA patients and therefore cannot be used to predict of AA.

Research into the appendix microbiome and the new biomarkers NGAL and LRG1 in blood serum and LRG1 in urine may provide a better understanding of complicated acute appendicitis in terms of etiopathogenesis and early diagnostic accuracy, as well as timely

diagnosis of disease severity and possible disease prognosis. It should be emphasised that the results of the study in Latvia could improve the quality of medical care in other countries.

The obtained results can also contribute beyond the borders of Latvia, as they have been published in international databases, as well as the text of the doctoral thesis is in English, so that other colleagues who are interested in this topic can familiarise themselves with the researched material and it is possible to introduce changes in their daily practice in connection with in paediatric patients with acute appendicitis.

Keywords: acute appendicitis, acute complicated appendicitis, acute uncomplicated appendicitis, surgery, paediatric patients, leucine-rich alpha-2 glycoprotein 1 (LRG1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin-6 (IL-6), *Escherichia coli*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*

Anotācija

Kompleksa klīniska, molekulārbioloģiska un mikrobioloģiska izpēte bērna vecuma akūta komplicēta un nekomplicēta apendicīta gadījumā

Visā procesa laikā, sākot no diagnostikas līdz efektīvai ārstēšanai apendicīts ir izaicinoša diagnoze bērna vecuma pacientiem. Ņemot vērā neķirurģiskās ārstēšanas metodes pielietošanu akūta apendicīta (AA) pacientiem bērna vecumā, viena no svarīgākajām neatliekamās bērnu ķirurģijas problēmām ir akūta nekomplicēta apendicīta (AnA) un akūta komplicēta apendicīta (AkA) nodalīšana ārstēšanas sākuma periodā, jo AkA liecina par novēlotu diagnostikas procesu un prasa tikai neatliekamu ķirurģisku ārstēšanu. Latvijā AkA sastopams vairāk nekā 35 % gadījumu. Mūsu līdzšinējie pētījumi liecina, ka VSIA BKUS līdz pēdējam laikam, pie tendences kopējam operēto AA gadījumu skaitam saglabāties iepriekšējā līmenī, AkA skaits pieaug.

Šodien eksistējošās AkA diagnostikas problēmas rada nepieciešamību meklēt jaunus agrīnās diagnostikas indikatorus pacientiem bērna vecumā, lai samazinātu komplikāciju attīstības biežumu, kā arī novērstu letalitātes riskus. Pirmais izaicinājums AA diagnostikā ir tā atšķiršana no citām neapendicīta (jeb nAA) diferenciāldiagnozēm. Pašlaik AA diagnostika ir atkarīga no klīniskās atrades, rūpīgas anamnēzes ievākšanas, un Alvarado skalas, kura pēdējā laikā tiek bieži pielietota un ir augsti vērtējams AA diagnostikas palīglīdzeklis, tomēr tai trūkst adekvātas jutības un specifiskuma, kā arī no pacientu (un/vai pacienta aizbildņu) sadarbības un komunikācijas spējām. Tiek izmantotas arī citas diagnostikas metodes, piemēram, datortomogrāfija (DT) un diagnostikā laparoskopija, bet šo metožu negatīvie aspekti samazina to diagnostikās vērtības produktivitāti (Podany et al., 2017). Ultrasonogrāfijas un asinsainas parametru (leikocitoze un paaugstināts C-reaktīvais olbaltums (CRO)) kā diagnostikas rīku izmantošana ir samazinājusi diagnostiskās laparoskopijas nepieciešamību, taču, neskatoties uz to, pašreizējais negatīvās apendektomijas biežums joprojām ir 1–40 % (Maloney et al., 2019). Savukārt šobrīd pieejamie biomarķieri ir neprecīzi un to novēlotā reakcija samazina neatliekamās palīdzības ārstu un bērnu ķirurgu iespējas sniegt savlaicīgu un potenciāli efektīvu terapiju. Tādējādi šī pētījuma mērķis, pirmkārt, ir atrast produktīvāku risinājumu savlaicīgai un efektīvai AA diagnostikai, nodalot AA un nAA.

Jau zināms, ka AkA ir sarežģītas izcelsmes aklās zarnas tārpveida piedēkļa iekaisums. Lai gan tradicionāli par AA izsaucēju tiek uzskatīta piedēkļa lūmena obstrukcija, nesenos pētījumos gūtie pierādījumi liecina, ka slimība, iespējams, attīstās tiešas specifisku patogēnu mikroorganismu invāzijas dēļ. To starpā tiek minēta arī *Yersinia enterocolitica* (Fernandes et al., 2020). Dažos agrākos pētījumos ir apstiprināts polimikrobiāls process, tomēr galveno

slimības izsaucēju un tā izcelsmi noteikt nav izdevies (Rogers et al., 2016); (Salö et al., 2017); (Bhattacharya et al., 2022); (Camacho-Cruz et al., 2022). Šis fakts ir palielinājis ekspertu un speciālistu interesi, kā arī pētījumu skaitu par antibakteriālo terapiju kā neinvazīvu metodi AA ārstēšanai. Ņemot vērā, ka mūsdienās palielinās neinvazīvas antibakteriālās terapijas izmantošana apendicīta ārstēšanai, šī pētījuma mērķis ir noskaidrot, kuri mikroorganismi ir sastopami pie AA izraisīšanas pētījumā iekļauto pediatrisko pacientu grupās (t. i., AkA un AnA grupās). Konkrētu mikroorganismu izplatība katrā no šīm grupām ir noteicošais faktors atšķirīgām antibakteriālās ārstēšanas stratēģijām, tāpēc šī pētījuma vēl viens mērķis ir noteikt, kuri mikroorganismi prevalē AnA un AkA grupās, kā arī pārbaudīt šo konkrēto mikroorganismu jutību un rezistenci un izvērtēt iespējamo antibakteriālo līdzekļu ārstēšanas efektivitāti.

Iespējamo biomarķieru apjoms, ko var izmantot akūta apendicīta diagnosticēšanas procesā, kā arī koncentrēšanās uz imunoloģiskajiem mehānismiem palielinās, taču pagaidām neviens no šiem biomarķieriem vēl netiek klīniskajā praksē plaši izmantots (Selleck et al., 2017). Viena optimālā biomarķiera atrašana varētu sagādāt lielas pūles, taču kombinācija ar pacienta medicīnas vēsturi un klīniskām atradēm varētu uzlabot diagnostikas kvalitāti un ātrumu, kas rezultēsies ar samazinātu komplikāciju risku, kā arī ar samazinātām slimnīcas kopējām izmaksām un, protams, izvairīšanos no nevajadzīgiem attēlveidošanas izmeklējumiem un ķirurģiskām operācijām.

Šis ir prospektīvs kontrolētu grupu pētījums laika posmā no 2017. līdz 2020. gadam, kur pacienti tika iedalīti 3 grupās: AkA (akūts komplicēts apendicīts), AnA (akūts nekomplicēts apendicīts) un Ktr (kontroles grupa). No 153 pacientiem 97 bija AA un 56 bija Ktr grupā. Iegūtie rezultāti parāda, ka urīna LRG1 (*leucine-rich alpha-2 glycoprotein 1*) ir precīzs marķieris AA diagnozes apstiprināšanai. Seruma un urīna LRG1 koncentrācija ir noderīga, lai noteiktu AA smagumu (t. i., AkA vai AnA). Biomarķieris NGAL (*neutrophil gelatinase-associated lipocalin*) asins serumā ievērojami palielinās 0. dienā un to būtu nepieciešams izmantot akūtu vēdera sāpju diferenciāldiagnostikā. Specifisko īpašību dēļ CRO un seruma IL-6 (*interleukin-6*) ir nespecifiski biomarķieri, taču tos var turpināt izmantot AA diagnostikas procesā un AkA un AnA diferencēšanā.

Pseudomonas aeruginosa biežāk tiek konstatēta AkA gadījumā un pēc jutības pārbaudes tā ir uzņēmīga pret cefalosporīnu grupas antibiotiķiem, piemēram, ceftazidīmu, tomēr *Pseudomonas aeruginosa* ir fenotipiska rezistence pret cefotaksīmu, tāpēc tas būtu jāizslēdz no AkA empīriskās ārstēšanas vadlīnijām. Lai panāktu sinerģisku efektu un novērstu antibakteriālo līdzekļu rezistences attīstību, ārstēšanas stratēģijā būtu jāiekļauj antibakteriālie līdzekļi ar dažādiem darbības mehānismiem. Šajā pētījumā ESBL (paplašināta spektra

beta-laktamāzes) veidojošo mikroorganismu sastopamība bija zema. *Yersinia enterocolitica* seruma antivielas AA pacientiem netika atklātas, tāpēc tās nevar tikt izmantotas AA prognozēšanai.

Pētot aklās zarnas tārpveida piedēkļa mikrobiomu un jaunus asins seruma NGAL un LRG1 un urīna LRG1 biomarķierus, varētu tikt sasniegta pilnvērtīgāka komplicēta akūta apendicīta etiopatoģenēzes izpratne un agrīnā diagnostiskā precizitāte, kā arī savlaicīgi noteikts slimības smagums un iespējamā slimības prognoze. Jāuzsver, ka pētījuma rezultāti Latvijā varētu uzlabot ārstnieciskās palīdzības kvalitāti pie salīdzinoši zemās sociāli ekonomiskās situācijas valstī.

Iegūtie rezultāti var sniegt ieguldījumu arī ārpus Latvijas robežām, jo ir publicētas starptautiskās datu bāzēs, kā arī promocijas darba teksts ir angļu valodā, lai citi kolēģi, kas ir ieinteresēti šajā tēmā, var iepazīties ar izpētīto materiālu un iespējams ieviest izmaiņas savā ikdienas praksē saistībā ar akūtu apendicītu pediatriskiem pacientiem.

Atslēgvārdi: akūts apendicīts, akūts komplicēts apendicīts, akūts nekomplicēts apendicīts, ķirurģija, pediatriski pacienti, *leucine-rich alpha-2 glycoprotein 1 (LRG1), neutrophil gelatinase-associated lipocalin (NGAL), interleukin-6 (IL-6), Escherichia coli, Pseudomonas aeruginosa, Yersinia enterocolitica*

Table of Contents

Abbreviations

AA	Acute appendicitis
AcA	Acute complicated appendicitis
AuA	Acute uncomplicated appendicitis
AUC	Area under the receiver operating characteristic curve
B-cells	B lymphocytes
CCUH	Children's Clinical University Hospital
CRP	C-reactive protein
CT	Computed tomography
Ctr	Control group
DDST	Double disk synergy test
ED	Emergency department
ESBL	Extended spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
IL-1β	Interleukin-1 beta
IL-22	Interleukin-22
IL-6	Interleukin-6
IQR	Interquartile ranges
LRG1	Leucine-rich alpha-2 glycoprotein 1
LRR	Leucine-rich repeat
Mdn	Median
nAA	Non-appendicitis
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
SoB	Study on biomarkers
SoMaS	Study on microbiota and susceptibility
Th17	T helper 17 cells
TNF-α	Tumour necrosis factor alpha
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 were set to achieve the aim of the study:

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 for antibacterial therapy in cases of AuA and AcA.

1 Literature overview

Acute appendicitis (AA) is one of the most common paediatric abdominal diseases. It requires surgery and, despite advances in diagnosis and treatment, is still predominantly based on typical clinical findings and patient history, and a unified understanding of the aetiology and pathogenesis of appendicitis is still lacking (Essenmacher et al., 2018); (Snyder et al., 2018). Acute appendicitis is the most common indication for emergency abdominal surgery in children and requires prompt evaluation and stage recognition to avoid morbidity and mortality (Hosseinpour, Ahmadi, 2016). Acute appendicitis can lead to abscess, peritonitis, sepsis, ileus or death due to delayed diagnosis and treatment (Podany et al., 2017); (Hong et al., 2020).

1.1 Epidemiology

The incidence of appendicitis in Western countries ranges between 100 and 150 cases per 100,000 person-years. In North America, for example, there were 378,614 cases of appendicitis per population in 2015, and the incidence there has been steadily decreasing since the 1990s. In the Baltic States, the incidence of appendectomy between 2005 and 2013 was 143–200 operations per 100,000 person-years across all age groups (Ferris et al., 2017). The incidence specifically of paediatric appendicitis in the Scandinavian and Baltic countries from 2004–2014 decreased to 80.7–120.8 cases per 100,000 person-years (Rautava et al., 2018).

1.2 Anatomy, physiology, and pathogenesis

The physiology of the appendix is also not fully understood. It is known that the vermiform appendix plays a role in the development and maturation of the immune system (Almaramhy, 2017). The involvement of microbes in the pathogenesis of appendicitis is not fully understood, but recent research suggests that the appendix acts as a microbiota reservoir in the gastrointestinal tract. It is thought to ensure repopulation of the microbiota during acute illness, when the gastrointestinal tract is colonised by pathogens such as acute gastroenteritis, and following antibacterial treatment (Heather et al., 2017).

1.3 Clinical features and diagnostics

Diagnosis of AA currently relies on typical clinical findings and anamnestic evidence, despite the development of various diagnostic techniques. The Alvarado score is a commonly used tool for grading AA symptoms, but it lacks specificity and sensitivity (Almaramhy, 2017). Although appendicitis is particularly common in children, the assessment of the clinical history

is often hampered by a lack of cooperation and fluency (Almaramhy, 2017). It is particularly difficult to collect the patient history of young children, who may be less cooperative and a less accurate sources of information. To promote accuracy, assessment methods such as computed tomography (CT) and diagnostic laparoscopy are used, but these are still time-consuming, costly, and invasive (e.g. CT-radiation increases the long-term risk of cancer) [46]. Current diagnostics such as leucocytosis, increased serum CRP, and abdominal ultrasound (US) imaging have helped to reduce the frequency of diagnostic laparoscopy (Pedram et al., 2019). Laparoscopy is considered a diagnostic method for abdominal pain of unclear aetiology and suspected appendicitis. Rates of negative appendectomy in children range from 1 % to 40 % in the literature (Maloney et al., 2019).

Ultrasound can be used to diagnose AA and, in most cases, can even differentiate between acute uncomplicated appendicitis (AuA) and acute complicated appendicitis (AcA). However, this all depends on the skill of the radiologist or sonographer (Rawolle et al., 2019). This has stimulated the search for a non-invasive strategy to reduce these error rates, which has led to the introduction of novel biomarkers being introduced for both the assessment and detection of appendicitis (Hodge et al., 2021)

Multiple studies have revealed the success of the efficient diagnostic scheme offered by inflammatory biomarkers as a non-invasive analysis, increasing the accuracy and speed of diagnosis and dramatically reducing healthcare costs (Hodge et al., 2021); (Hajibandeh et al., 2021). As immunological pathways are better understood, more biomarkers have been proposed as potential diagnostic tools, but none are in widespread use. Compared to IL-6 and CRP, no other biomarkers have been shown to be effective in the diagnosis of appendicitis. The prospect of a biomarker with even higher accuracy rate than those currently being investigated is exciting.

The introduction of novel biomarkers as part of the diagnostic criteria offers a non-invasive method that can yield similar information and diagnostic accuracy.

1.4 Leucine-rich alpha-2 glycoprotein (LRG1)

A potential new biomarker of inflammation, also produced by neutrophils, is leucine-rich alpha-2 glycoprotein 1 (LRG1). It is thought to not only have a particularly important and rapid diagnostic precision ratio, but also to determine specificity in the development of acute appendicitis with drug-independent serum levels (Naka, Fujimoto, 2018); (Tintor et al., 2023) Although its full mechanism of action is still unclear, LRG1 is thought to play a role in the activation and chemotaxis of neutrophils as they enter areas of inflammation (Naka, Fujimoto,

2018); (Camilli et al., 2022). LRG1 is a 50kD membrane-associated acute phase protein of the leucine-rich repeat (LRR) motif, consisting of 312 amino acids – 66 of which are leucine (Naka, Fujimoto, 2018); (Camilli et al., 2022). LRG1 is produced and secreted by hepatocytes, so it is not strictly dependent on any of these cells, and is upregulated in acute phase responses of microbial infections at inflammatory sites (Naka, Fujimoto, 2018). Its normal serum level is thought to be 21–50g/mL (Zhang et al., 2018).

Numerous pro-inflammatory markers such as IL-6, IL-1β, IL-22, TNF-α, and lipopolysaccharides stimulate the transcription of LRG1; therefore, it is not dependent on a single stimulating factor. Another peculiar facet of LRG1 is the remarkably increased concentration at the local site of inflammation, which may differentiate infections on the basis of marked LRG1 deposition (Camilli et al., 2022). The current diagnostic issues of AcA highlight the need to find new early diagnostic indicators for paediatric patients in order to reduce the incidence of complications.

1.5 Interleukin-6 (IL-6)

Interleukin-6 (IL-6) is a highly multifunctional inflammatory marker of inflammation that is strongly dependent on TNF-α and IL-1β for its production. Its functions are very diverse, including initiation of acute phase protein synthesis in the liver, activation of haematopoiesis, activation of B cells, and participation in the formation of T helper 17 cells (Th17) (Wu et al., 2016). IL-6 is found at high levels in patients with sepsis. Recent literature has shown that this marker is an ideal biomarker for bacterial infections and could serve as an early rapid diagnostic tool for clinically suspected appendicitis (Wu et al., 2016).

1.6 Neutrophil gelatinase-associated lipocalin (NGAL)

Neutrophils are the primary responders to inflammation, and if their numbers in the bloodstream are increased, this could also mean an increase in neutrophil gelatinase-associated lipocalin (NGAL). NGAL is thought to increase in the bloodstream along with neutrophils in the respiratory and gastrointestinal tracts, and in the renal system when epithelial tissue is damaged. Serum NGAL levels appear to increase in correlation with epithelial damage under stress, e.g. processes such as inflammation, infection and ischaemia (Bakal et al., 2016); (Selleck et al., 2017). Thus, theoretically, both IL-6 and NGAL serum levels would be higher in AcA than in AuA, where organ tissues are less stressed. The focus on immunological pathways is increasing, as is the number of proposed biomarkers, although none has yet

achieved widespread use. Compared to IL-6 and CRP, no other biomarkers have been shown to be effective in the diagnosis of AA.

1.7 Treatment

Historically, the only effective treatment for appendicitis and prevention of septic complications has been surgery, namely, an appendectomy, which has been practised for over 130 years (Rogers et al., 2016). This view has been challenged in recent years as conservative treatment with antibiotics has replaced surgery (Coccolini et al., 2018); (Becker et al., 2018). Clinical research has demonstrated the efficacy of antibacterial treatment, yet 27 % of patients still require surgery each year (Kakar et al., 2020).

The therapeutic plan for acute appendicitis in children has evolved to favour non-surgical antibacterial treatment over surgical treatment. Complicated appendicitis is the most common cause of intra-abdominal infection in children (Aiyoshi et al., 2021). To reduce the risk of postoperative complications in complicated appendicitis, such as wound infections and intra-abdominal abscesses, antibiotics are included in treatment protocols. However, there is no consensus on the optimal choice of antibiotic regimen for acute appendicitis in children. Furthermore, the most appropriate regimen may change depending on the geographical distribution of species of pathogenic and opportunistic pathogens and their antimicrobial resistance. Therefore, it is important to clinically assess the aetiopathology of paediatric appendicitis (simple and complex) and to analyse the antimicrobial susceptibility of its causative agents (Schulin et al., 2017); (Bhattacharya et al., 2022).

The recent interest in and evidence for non-surgical treatment with antibiotic therapy leads to the recurring problem of differentiating AuA from AcA when presenting to the Emergency Department (ED) at the start of conservative management, as complicated cases indicate a delay in diagnosis and require emergency surgery. Rapid confirmation of AA is hampered by a number of factors, including atypical presentation and multiple differential diagnoses, making complications more likely. These cases account for more than 35% of all AA cases in Latvia. Our current research has shown that the total number of AA cases treated surgically at the Children's Clinical University Hospital has not changed, but the incidence of AcA has increased (Kakar et al., 2021). The increased use of conservative treatment requires evaluation of algorithms for antibacterial treatment, as these may vary among clinical institutions.

2 Materials and methods

2.1 Study setting and study population.

The research was designed as a prospective, single-centre, randomised, controlled cohort study including children aged 7 to 18 years who were admitted to the Children's Clinical University Hospital due to acute abdominal pain and signs and symptoms consistent with possible appendicitis. All patients were examined to confirm or exclude this diagnosis. Preoperative screening involved physical examination, complete blood count, abdominal ultrasound (US) and detection of serum values of C-reactive protein (CRP) and interleukin-6 (IL-6).

All procedures performed in this study involving human participants followed the ethical standards of the institutional and/or national research committee, and the studies met the requirements of the Patient's Data Protection Law and the principles of the Declaration of Helsinki. The Ethics Committee's approval was obtained from both the Children's Clinical University Hospital and Rīga Stradiņš University (reference number: SP-37/2018 and 21/27.04.2017, respectively) between January 2017 and 2020, during which the research was conducted. Clinical data collected prior to surgery included patients' age, sex, and current medical history.

The study group included patients with confirmed AA who were treated with appendectomy, either laparoscopic or conventional laparotomy. Patients with suspected appendicitis, but with previous abdominal surgery, pregnancy, and chronic medical conditions that could potentially affect the renal, gastrointestinal, or respiratory systems (e.g. inflammatory bowel disease, chronic pancreatitis, acute kidney injury, and immunosuppressed patients) were excluded. This exclusion was due to the limited study group size of approximately 150 patients. The control group (Ctr) included patients without a suspected inflammatory process in the respiratory, renal or gastrointestinal tract, but who were admitted to the emergency department for the treatment of different types of traumas (e.g., fractures, dislocations, contusions, muscle tears, testicular torsion, blunt abdominal trauma).

Microbiological culture swabs were taken intraoperatively from the appendix and peritoneal cavity. Depending on the intraoperative and bacteriological findings, two groups were established – acute complicated appendicitis (AcA) and acute uncomplicated appendicitis (AuA).

The paediatric surgery team supervising patients with appendicitis received a written consent form from the caregiver and assent from the patient if they were 13 years of age or older. The consent form informed the patient and the caregiver about the research objective and

methodology used to study the biological material. Informed consent was obtained from a parent of each individual participant included in the study. The size of the patient group was divided to be equal to limit the assumption of variances such as the Levene's test.

The consent and assent concerned the research objective and methodology used for investigating the biological material (Abdurrazzaaq et al., 2018); (European Committee on Antimicrobial Susceptibility Testing (EUCAST); (Clinical Breakpoints and Dosing of Antibiotics). A schematic image of the study is shown in Figure 2.1.

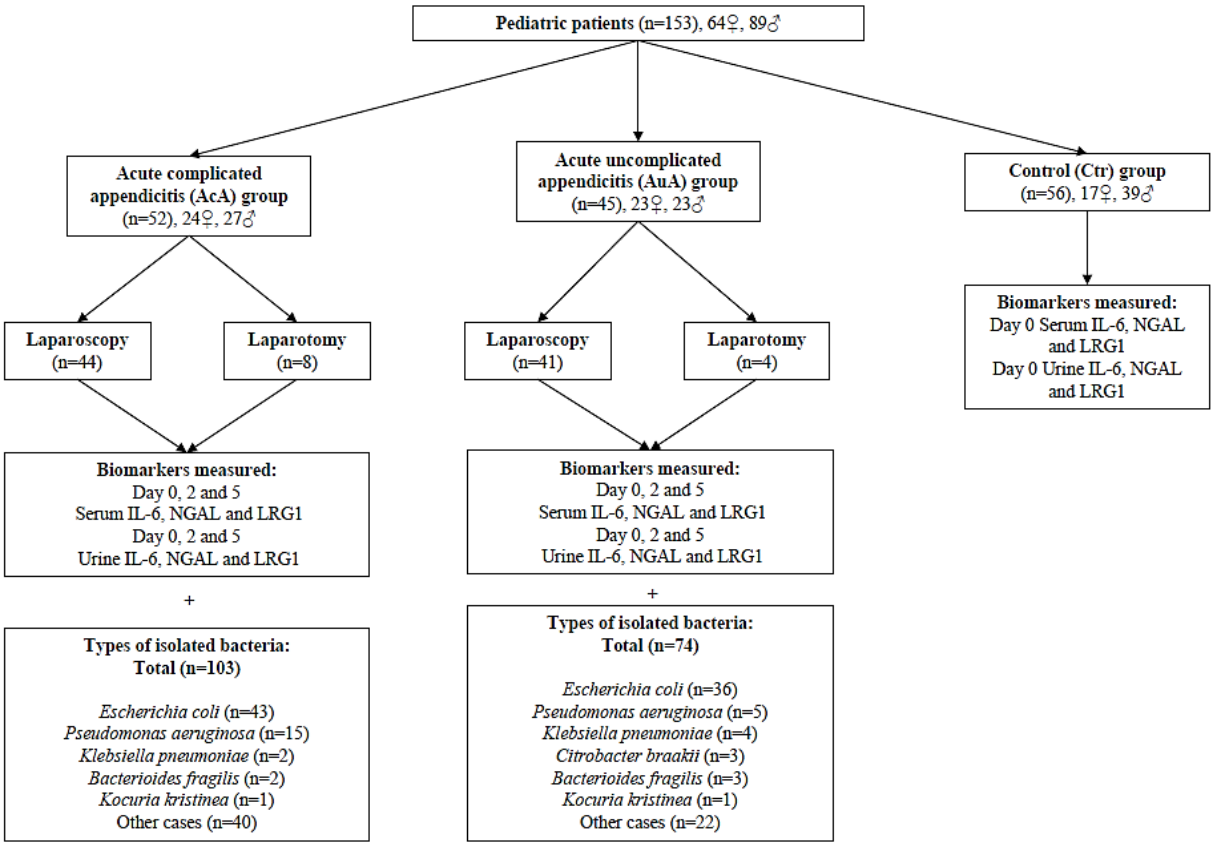


Figure 2.1 **Schematic image of the study**

2.2 Study protocol

Each patient's medical history, physical examination, Alvarado score, complete blood count, biochemical blood analysis, CRP and IL-6 were collected by the treating physician according to the hospital protocol (Nr. REK-052/01), and if the Alvarado score was six or more, a paediatric surgeon was consulted. All patients were examined to confirm or exclude this diagnosis. Depending on the need, preoperative screening involved physical examination, complete blood count, abdominal ultrasound (US), and detection of serum values of CRP and IL-6. Once appendicitis was confirmed and all inclusion criteria were met, a consent form was given to the patient and his/her caregiver for both the AA and control groups.

According to the diagnostic and treatment algorithm for AA at the Children's Clinical University Hospital (CCUH), Riga, the criteria for AuA are Alvarado score ≥ 7, CRP cut-off values 8.4 mg/L or IL-6 cut-off values 36.2 pg/ml and signs of AA shown in the ultrasound. The criteria for AcA from the ultrasound are Alvarado score ≥ 7, CRP cut-off values > 8.4 mg/L or IL-6 cut-off values > 36.2 pg/ml; signs of AA, and peritoneal irritation symptoms are also presented.

IL-6, NGAL and LRG1 concentrations were measured on days 0, 2 and 5 (baseline, second and fifth postoperative day respectively). Patients were operated on by the hospital's paediatric surgeons with the author present. Intraoperatively, a microbiological culture swab from the peritoneal cavity was collected. Patients were classified as AcA or AuA by the presence or absence of bacterial growth in the peritoneal cavity. After the removal of the appendix, an extra submucosal swab was taken to avoid bacterial dissemination from subsequent swabs. The appendix was dissected longitudinally and under sterile conditions, and swab samples were taken from the distal and proximal part of the appendiceal lumen. Each pair was placed in Amies medium for immediate transfer and subsequent bacterial culture (Rainer et al., 2017). They were cultured under aerobic and anaerobic conditions. Cultivation was performed on blood agar (Supplement, Oxoid, Hampshire, UK; Defibrinated Sheep blood E&O laboratories limited, Falkirk, Scotland), MacConkey (Oxoid, UK) and trypticase soy (Oxid, UK) agar. Bacterial identification was performed using the VITEK2 analyser (Biomerieux, Auvergne-Rhône-Alpes, France).

The primary outcome was a difference in biomarker levels between AA and the Ctr and, secondarily, whether there was a difference in biomarkers between AcA and AuA.

2.3 Serum Biomarker Collection and Analysis – SoB

2.3.1 Sample collection, transport, and storage

On the day of hospital admission, blood and urine samples were taken from all patients. The minimum amount of blood per patient for IL-6, NGAL and LRG1 was 300 µl, 100 µl and 300 µl respectively. After centrifugation, serum was collected and stored at −80 °C. Midstream, clean-catch urine specimens (minimum amount of IL-6 200 µl; NGAL 100 µl; LRG1 200 µl) were collected and centrifuged at 2000 rpm for 10 minutes at 4 °C to remove cell debris from the urine. The supernatant was stored at −80 °C till further analysis.

Serum and urine samples were assayed for IL-6, NGAL and LRG1 preoperatively, and on days 2 and 5 postoperatively.

2.3.2 Measurement of LRG1, NGAL and IL-6 levels

Commercially available kits were used to determine biomarker levels in serum and urine: human LRG1 enzyme-linked immunosorbent assay (ELISA) kit (Catalogue No. NBP2-60577, Novus Biologicals, USA), human lipocalin-2/NGAL Quantikine® ELISA, and human IL-6 Quantikine® ELISA (R&D Systems, Minneapolis, MN, USA). All procedures performed with these concentrations were performed according to the instructions of these kits. IL-6 (Immunoassay Control Group 1 QC01-1, R&D System Inc., Minneapolis, MN, USA) and NGAL (Immunoassay Control Set QC115, R&D System Inc., Minneapolis, MN, USA) controls were used to ensure experimental quality. All the samples were tested in three separate wells and results were obtained within approximately 2–4 hours. All of these kits used a quantitative sandwich enzyme immunoassay technique. Biomarkers from patient samples were captured on pre-coated antibodies on specific polyclonal wells and then recognised by the detection antibodies. After washing away the unbound materials, a peroxidase chromogen substrate was added to measure colour intensity. The LRG1 kit required the addition of streptavidin-peroxidase conjugate before adding substrate solution. The minimum detectable LRG1 serum level was 0.313 ng/mL. Results were measured at 450 nm with wavelength correction at 570 nm. Values obtained in ng/mL were converted to μg/mL for further calculations.

2.3.3 Urine Biomarkers Collection and Analysis

The same kit and instruction manual procedure were employed for urine analyses. Midstream, clean-catch urine specimens of at least 200 μl were collected in a sterile cup on admission, and subsequently for the AA participants on postoperative days 2 and 5. Most AA patients had their Day 0 urine sample collected during surgery through a urinary catheter. Urine samples were centrifuged at 2000 rpm for 10 minutes at 4 ℃ and then these supernatants were stored at −80 ℃ before analysis. The following day, the samples were transported to the laboratory for processing. The same steps of ELISA as for the serum biomarkers were performed on these samples.

2.4 Microbiological studies and evaluation – SoMaS

Tests were conducted on antibacterial susceptibility and evaluation on the subsequent results was in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), specifically “Clinical breakpoints and dosing of antibiotics” (Version 10.0, 2020).

Overnight cultures were suspended in physiological saline to 0.5 McFarland units (McFarland Densitometer DEN-1, Biosan, Latvia). The suspension was inoculated on Mueller-Hinton agar (Oxid, UK). Selected antibiotics were placed on the inoculated plates and included ceftazidime 10 μg, ampicillin 10 μg, cefotaxime 5 μg, meropenem 10 μg, imipenem 10 μg, amikacin 30 μg, gentamicin 10 μg, ciprofloxacin 5 μg, chloramphenicol 30 μg, ertapenem 10 μg, amoxicillin+clavulanic acid 30 μg, and piperacillin+tazobactam 36 μg (Liofilchem, Italy). The plates were incubated at +35 ± 1 °C temperature for 18 ± 2 hours. A double disk synergy test (DDST) was used to confirm extended spectrum beta-lactamase (ESBL). Disks containing cephalosporins (cefotaxime, ceftazidime) were applied to plates next to a disk with clavulanic acid (amoxicillin+clavulanic acid). A positive result was indicated if the inhibition zones around any of the cephalosporin disks were augmented in the direction of the disk containing clavulanic acid. Results were evaluated by measuring the zone of inhibition, and resistance was interpreted in accordance with the EUCAST breakpoints.

Haematoxylineosin staining was performed, and the grade of inflammation was assessed, differentiating gangrenous and phlegmonous appendicitis. Serodiagnosis of *Yersinia enterocolitica* was performed using indirect (passive) haemagglutination.

2.5 Statistical analysis

Microsoft Excel 2016 and (Microsoft, USA) IBM SPSS Statistics 27 (IMB, USA) were used for statistical analyses, and all data were validated by a statistical analyst to ensure accuracy. Medians and interquartile ranges (IQRs) were used to express the results for quantitative data. 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 distributions. Pearson Chi-square and Fisher Exact Tests were used for nominal variables to determine associations between them.

In the study of diagnostic biomarkers for AA, a receiver operating characteristic (ROC) curve was generated by plotting the false-positive fraction versus the true-positive fraction for each possible cut-off score, and the area under the ROC curve (AUC) was calculated to determine the clinical significance of the biomarkers and their diagnostic value for appendicitis.

Binary logistic regression is 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 the biomarkers were assessed using receiver operating characteristic (ROC) curve and binary logistic regression models. Two different models were analysed – AA vs Ctr and AcA vs AuA. A p-value of < 0.05 was considered statistically significant.

3 Results

Samples were collected from 153 patients eligible for this research. 97 (63.4 %) were diagnosed with appendicitis and 56 (36.6 %) had no suspected infectious or inflammatory pathology. The age of the participants 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). Of these, 89 (58.2 %) were boys and 64 (41.8 %) were girls.

Suspected appendicitis 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 fluid were taken to determine the presence of bacterial growth in the peritoneal cavity, as the acute complicated (AcA) and uncomplicated appendicitis (AuA) were differentiated on the basis of bacterial growth. Patients with a positive culture from peritoneal cavity samples were classified in the AcA group, with 52 patients (53.6 %), and those with a negative culture were classified in the AuA group, with 45 patients (46.4 %).

3.1 Results – biomarkers

3.1.1 Demographics characteristics of the patients

Nine AuA (22.5 %) and 31 AcA (77.5 %) patients required a drainage tube placement. More than half of the patients (60.8 %) who required a drainage tube were diagnosed with AcA ($p < 0.001$). A simple comparison suggests that AcA had a slightly longer median postoperative hospital stay, six versus five days. The demographic characteristics of the patients are shown in Table 3.1.

Table 3.1

Demographic characteristic of the patients

Indicator	AuA	AcA	Ctr*	Total	p-value
	n = 45	n = 52	n = 56	n = 153	
Gender, n (%)					
Boy	22 (14.4)	28 (18.3)	39 (25.5)	89 (58.2)	0.081a
Girl	23 (15.0)	24 (15.7)	17 (11.1)	64 (41.8)	
Age, Mdn (IQR)	13.0 (10.0–15.0)	12.0 (9.0–14.0)	13.5 (10.3–15.0)	–	0.101c
Type of surgery, n (%)					
Laparoscopy	41 (91.1)	44 (84.6)	–	85 (87.6)	0.333a
Laparotomy	4 (8.9)	8 (15.4)	–	12 (12.4)	

Table 3.1 continued

Indicator	AuA	AcA	Ctr*	Total	p-value
	n = 45	n = 52	n = 56	n = 153	
Drainage tube, n (%)					
Yes	9 (20.9)	31 (60.8)	–	40	**< 0.001a**
No	34 (79.1)	20 (39.2)	–	54	
Length of hospital stay, days (IQR)	5 (4–6)	6 (4–9)	–	–	**0.002b**

AcA – Acute complicated appendicitis, AuA – Acute uncomplicated appendicitis, Mdn – Median, IQR – Interquartile range (Q1 – Q3), *a – Pearson Chi-square test, b – Mann-Whitney U-test, c – Kruskal-Wallis test

3.1.2 Preoperative and postoperative biomarker levels per appendix status

The preoperative baseline values of IL-6, NGAL and LRG1 are presented in Table 3.2, together with the levels on postoperative days 2 and 5. The lowest baseline level (Day 0) of all observed parameters was found in the control group (Ctr) without infectious disease, while the highest was observed in AcA. The drastic decrease in the levels of the biomarkers S-IL-6 and S-NGAL can be observed from Day 0 to Day 5, as the inflammation settles postoperatively, data are presented in Table 3.2.

Table 3.2

Preoperative and postoperative biomarker levels per appendix status

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

Table 3.2 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 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
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 Day 0 are included in this study

3.1.3 Serum IL-6 levels

The median serum IL-6 (S-IL-6) levels on Day 0 for the AuA, AcA and control groups were 22.57 pg/ml, 70.59 pg/ml, and 6.44 pg/ml respectively (Figure 3.1). 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, $p < 0.001$), and the distribution of S-IL-6 was statistically higher in AcA compared to AuA (pairwise comparison, $p = 0.007$) and in AuA compared with the control group (pairwise comparison, $p < 0.001$). A decrease in biomarker levels can be observed between Day 0 and Day 5, as the inflammation settles postoperatively.

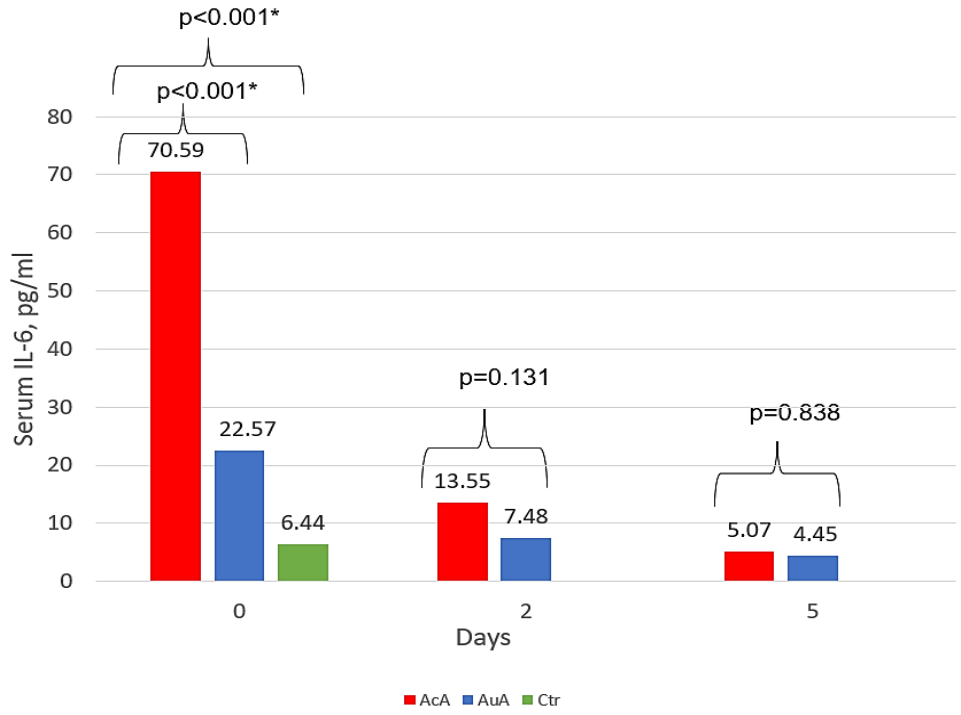


Figure 3.1 **Serum IL-6 levels on operative Day 0, postoperative Day 2 and postoperative Day 5 in patients with AcA, AuA and Ctr**

3.1.4 Urine IL-6 levels

The urine IL-6 (U-IL-6) samples were inconclusive and, thus, not specific enough to differentiate between AcA and AuA or between infectious disease and non-infectious disease (Figure 3.2).

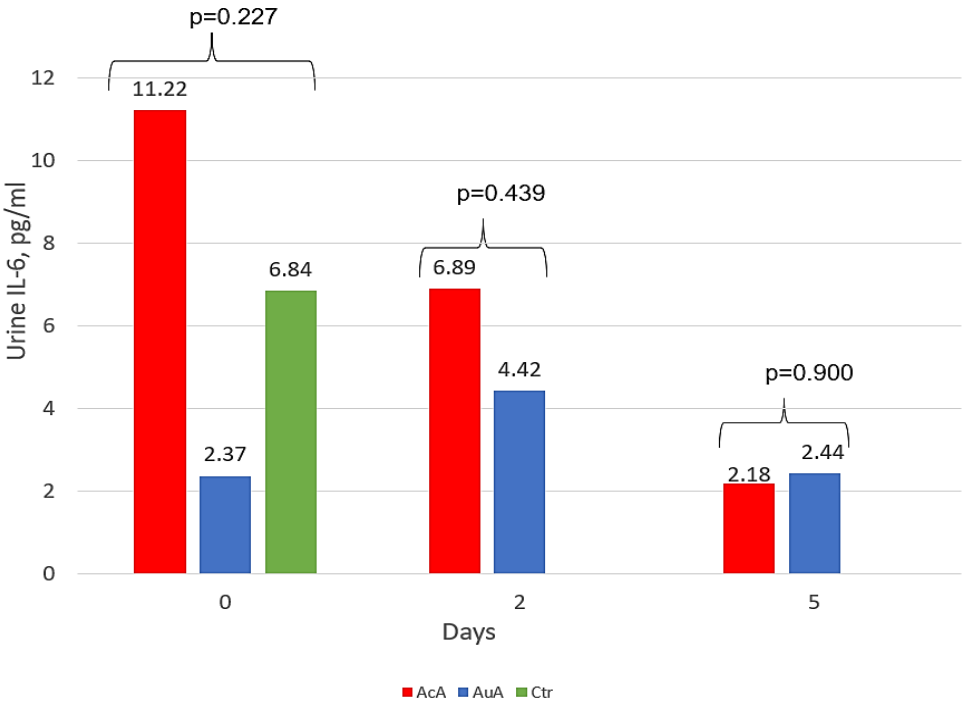


Figure 3.2 **Urine IL-6 levels on operative Day 0, second postoperative Day 2 and fifth postoperative Day 5 in patients with AcA, AuA and Ctr**

3.1.5 Serum NGAL levels

The median serum NGAL (S-NGAL) levels on Day 0 for AuA, AcA and Ctr were 128.20 ng/mL, 169.90 ng/mL, and 90.37 ng/mL, respectively (Figure 3.3). The distribution of S-NGAL on Day 0 was significantly different (Kruskal-Wallis tests, T stat = 19.04, $p < 0.001$) in AcA compared to Ctr (pairwise comparison, $p < 0.001$). S-NGAL values of AuA were higher than those of Ctr (pairwise comparison, $p = 0.087$). Thus, the S-NGAL values were the highest in AcA.

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 postoperative Day 5 decreased to 85.25 ng/mL in AcA and 69.8 ng/mL in AuA ($p = 0.220$).

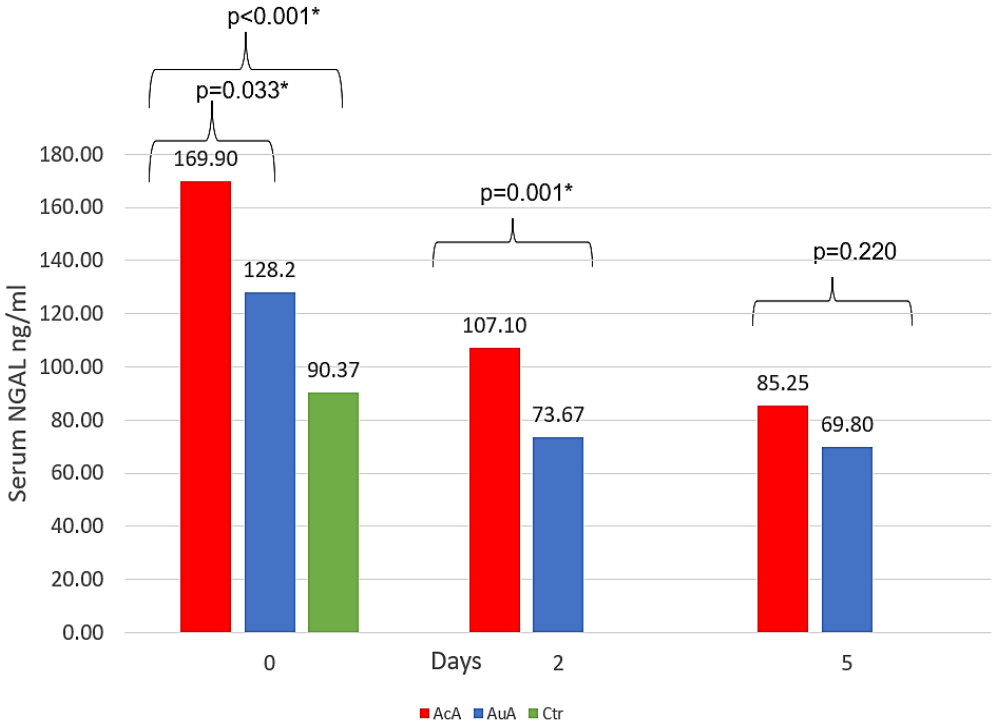


Figure 3.3 **Serum NGAL levels on operative Day 0, second postoperative Day 2 and fifth postoperative Day 5 in patients with AcA, AuA and Ctr**

3.1.6 Urine NGAL levels

The urine NGAL (U-NGAL) samples were inconclusive and thus not specific enough to differentiate between AcA and AuA or between infectious and non-infectious disease (Figure 3.4).

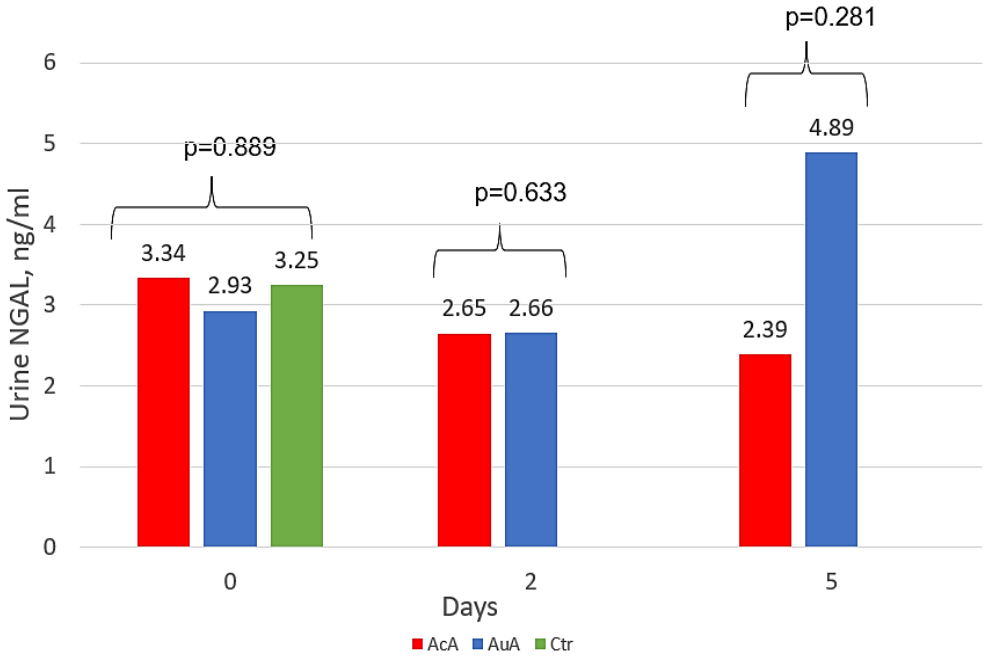


Figure 3.4 **Urine NGAL levels on operative Day 0, second postoperative Day 2 and fifth postoperative Day 5 in patients with AcA, AuA and the Ctr**

3.1.7 Serum LRG1 levels

The median serum LRG1 (S-LRG1) levels on Day 0 for AuA, AcA and Ctr were 70.56 µg/ml, 88.12 µg/ml, and 34.08 µg/ml, respectively (Figure 3.5). The Day 0 serum LRG1 median value is more than twice as high in AuA compared to Ctr, and almost three times as high in AcA. The distribution of serum LRG1 on Day 0 was significantly different (Kruskal-Wallis test, T stat = 88.30, $p < 0.001$) in AcA compared to Ctr (pairwise comparison, $p < 0.001$); equally, the distribution of S-LRG1 was significantly different in AuA compared to 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 the levels on admission to the ED ($p < 0.001$) (Table 3.2). Thus, these results suggest that S-LRG1, as a novel biomarker after appendectomy, correlates with patient recovery.

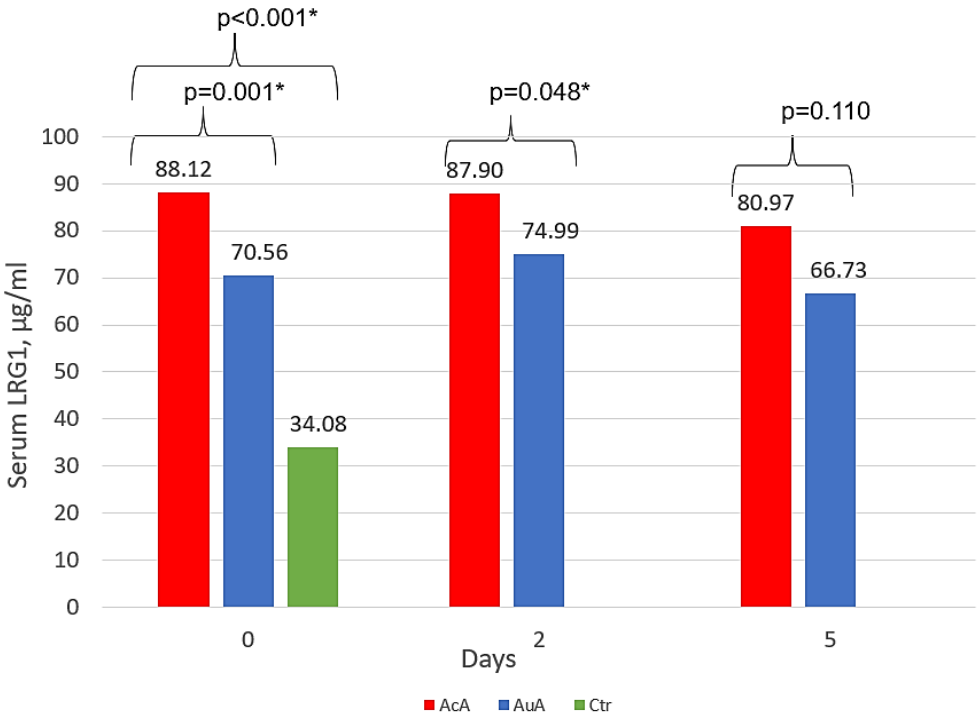


Figure 3.5 **Serum LRG1 levels on operative Day 0, postoperative Day 2 and postoperative Day 5 in patients with AcA, AuA and Ctr**

An additional assessment of the relationship between S-LRG1 concentration and disease severity in AA patients is shown in Figure 3.6, which shows that appendiceal mucosal inflammation correlates significantly with 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 comparing only AcA versus AuA.

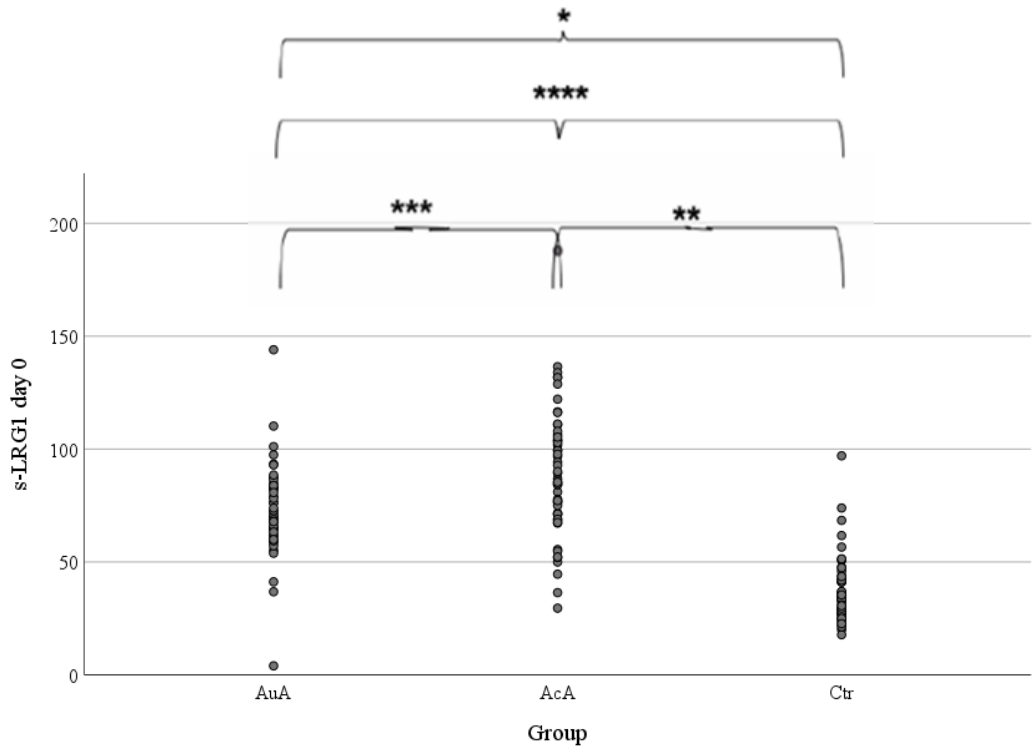


Figure 3.6 **S-LRG1 of each patient group**

S-LRG1 levels are increased in patients with AA (n = 97) compared to the control group (n = 56), **** $p < 0.001$. S-LRG1 could detect disease progress when analysing AcA (n = 52) and AuA (n = 45), *** $p = 0.001$. A significant difference was recognized when the control group was compared to AcA and AuA separately, * $p < 0.001$ and ** $p < 0.001$, respectively.

3.1.8 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 (Table 3.2). These differences are shown in Figure 3.7, where the 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 ($p < 0.001$, $p = 0.005$).

Urine LRG1 (U-LRG1) levels on postoperative Day 5 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 postoperative Day 5 ($p < 0.001$).

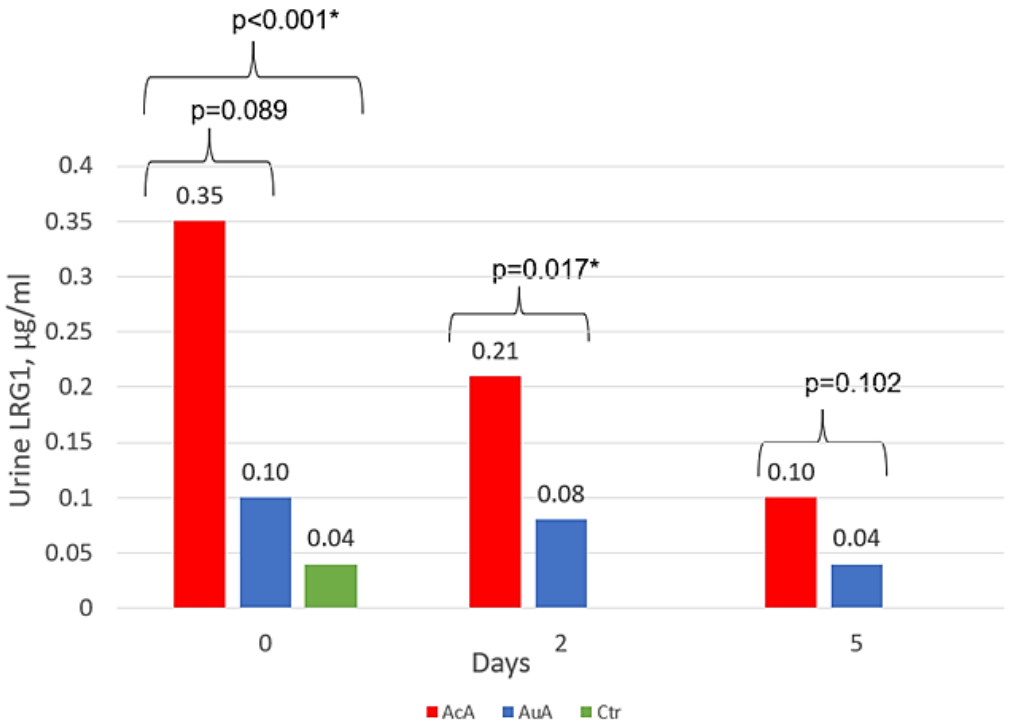


Figure 3.7 **Urine LRG1 levels on operative Day 0, second postoperative Day 2 and fifth postoperative Day 5 in patients with AcA, AuA and the Ctr**

Further assessment of whether U-LRG1 levels are associated with disease activity in patients with AA is demonstrated in Figure 3.8. This shows that appendiceal mucosal inflammation correlates significantly 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 ($p = 0.089$).

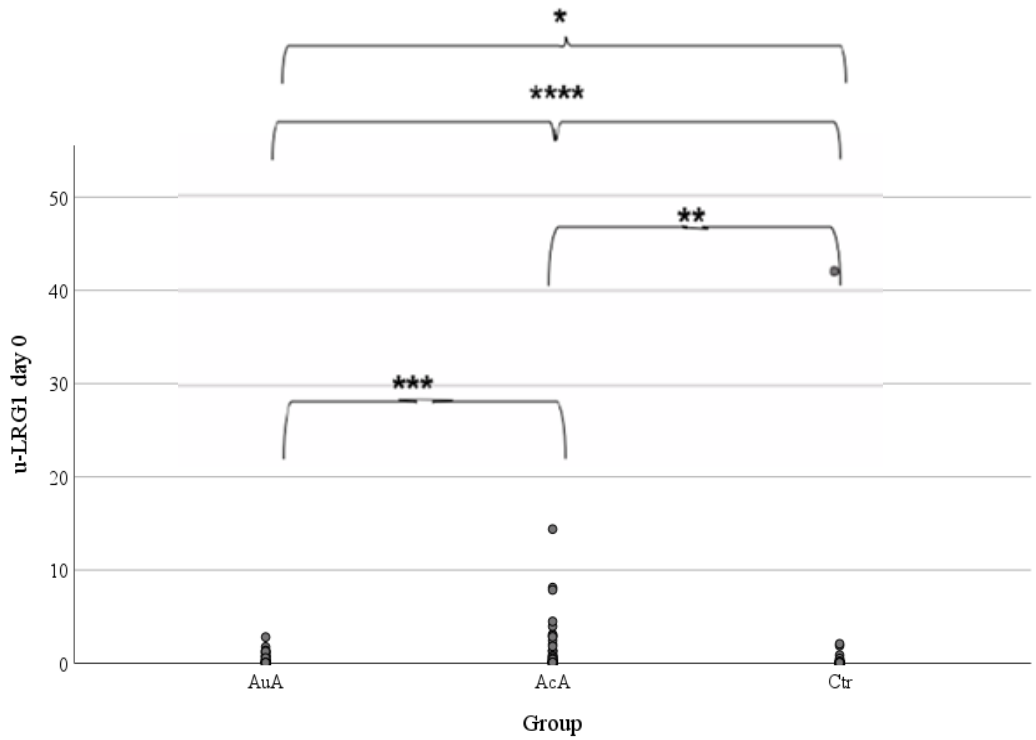


Figure 3.8. **U-LRG1 of each patient group**

U-LRG1 levels are increased in patients with AA (n = 97) compared to the control group (n = 56), **** $p < 0.001$. u-LRG1 was not able to detect disease progress when analysing AcA (n = 52) and AuA (n = 45), *** $p = 0.089$. A significant difference was recognized when the control group was compared to AcA and AuA separately; * $p < 0.001$ and ** $p = 0.005$, respectively.

U-LRG1 levels decreased to 0.10 μg/ml in AcA and 0.04 μg/ml in AuA on the fifth postoperative day ($p = 0.102$). U-LRG1 levels were significantly higher at the time of admission to the emergency department than on postoperative Day 5, ($p < 0.001$) (Table 3.2.). U-LRG1 concentrations dropped by more than 50 % (52/93 patients) after resection of the diseased appendix. Thus, these results suggest that U-LRG1, as a novel biomarker, correlates with improved patient recovery after appendectomy.

3.1.9 Comparison of serum and urine biomarker levels

The urine samples for all three biomarkers together were inconclusive and therefore not specific enough to differentiate between AcA and AuA (Table 3.3). When comparing AcA with AuA, a significant difference was seen between baseline (Day 0) S-IL-6, S-NGAL and S-LRG1 individually ($p < 0.001$, $p = 0.033$, and $p = 0.001$) (Table 3.3).

Table 3.3

Evaluating preoperative biomarker levels of AcA versus AuA

Biomarkers		AuA, ng or pg or µg/ml Median (IQR)	AcA, ng or pg or µg/ml Median (IQR)	p-value
Day 0				
Serum	IL-6	22.57 (11.15–42.21)	70.59 (25.06–300.92)	**< 0.001**
	NGAL	128.20 (81.44–184.50)	169.90 (104.95–258.15)	**0.033**
	LRG1	70.56 (62.64–83.43)	88.12 (71.12–106.13)	**0.001**
Urine	IL-6	2.37 (0.55–27.93)	11.22 (2.82–29.10)	0.115
	NGAL	2.93 (1.41–8.57)	3.34 (1.10–10.45)	0.739
	LRG1	0.10 (0.03–0.73)	0.35 (0.05–1.38)	0.089

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.

3.1.10 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 ($p < 0.001$), as shown in Figure 3.9, Figure 3.10, Figure 3.11, Figure 3.12, and Table 3.4.

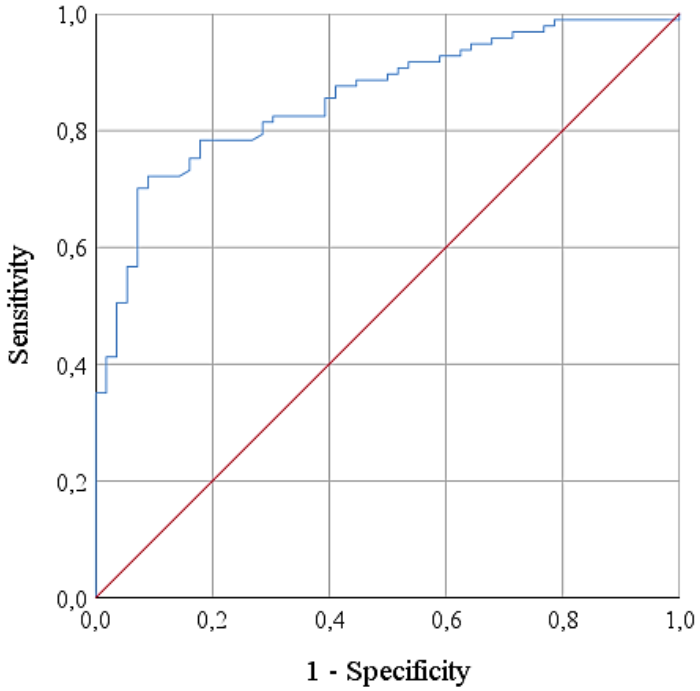


Figure 3.9 **ROC curve analysis of appendicitis (AcA and AuA) vs control group of serum IL-6**

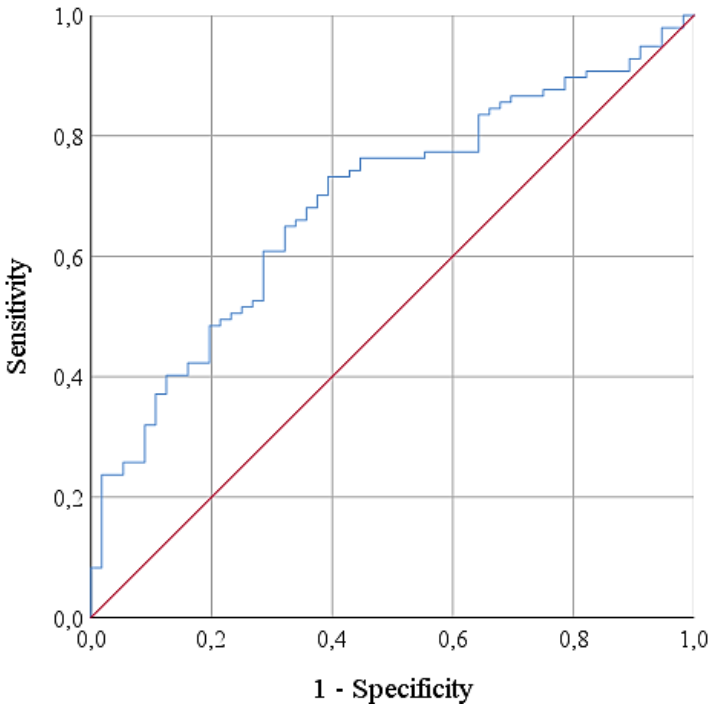


Figure 3.10 **ROC curve analysis of appendicitis (AcA and AuA) vs control group of serum NGAL**

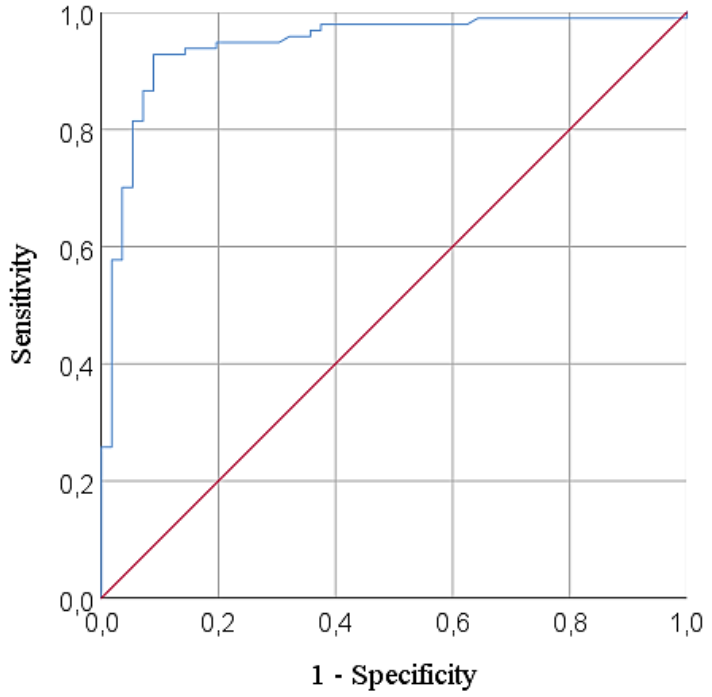


Figure 3.11 **ROC curve analysis of appendicitis (AcA and AuA) vs control group of serum LRG1**

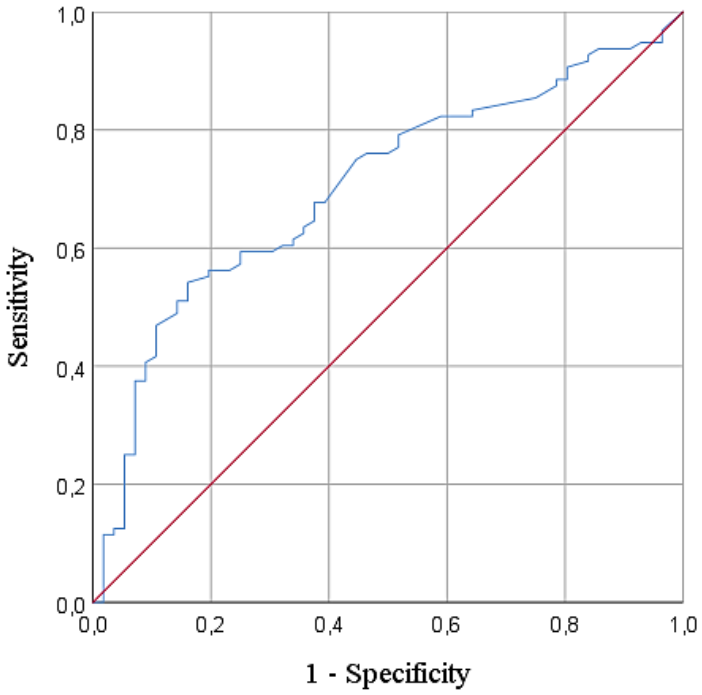


Figure 3.12 **ROC curve analysis of appendicitis (AcA and AuA) vs control group of urine LRG1**

Table 3.4

The ROC curves for biomarkers in appendicitis vs non-appendicitis patients

Biomarkers	AUC	95 % CI	p-value	Cut-off	Sensitivity	1-specificity	Specificity
IL-6 0	0.856	0.798–0.915	< **0.001**	20.25	0.719	0.089	0.911
NGAL 0	0.689	0.604–0.773	< **0.001**	103.75	0.729	0.393	0.607
LRG1 0	0.945	0.905–0.985	< **0.001**	51.69	0.938	0.089	0.911
U-LRG1 0	0.703	0.619–0.787	< **0.001**	0.175	0.542	0.161	0.839
CRP 0	0.851	0.790–0.931	< **0.001**	2.45	0.825	0.111	0.889

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 (Figure 3.13). The ROC curve for U-LRG1 showed an AUC of 0.703 (95 % CI 0.619–0.787) and for CRP an 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 had a higher sensitivity and specificity of 93.8 % and 91.1 % respectively.

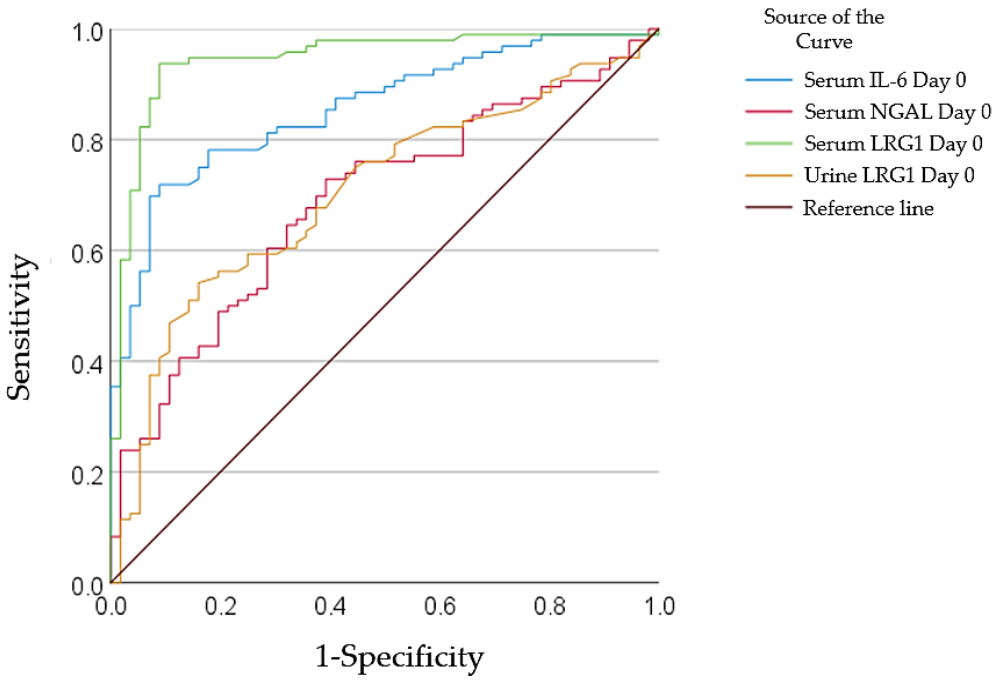


Figure 3.13 **The ROC curves demonstrate AUC 0.856 (95 % CI 0.798–0.915) for IL-6, AUC 0.689 (95 % CI 0.604–0.773) for S-NGAL, AUC 0.945 (95 % CI 0.905–0.985) for S-LRG1 and AUC 0.703 (95 % CI 0.619–0.787) for U-LRG-1**

The binary logistic regression shows that among the biomarkers taken on admission IL-6 and LRG1 were significantly associated with the diagnosis of appendicitis (Table 3.5).

Table 3.5

Binary logistic regression results and coefficient values used for this study

AA vs Ctr						
Covariates	**B**	**S.E.**	**Wald**	**p-value**	**OR**	**95 % CI for OR**
IL-6 0	0.072	0.024	9.058	0.003	1.075	1.025–1.126
NGAL 0	0.002	0.004	0.332	0.564	1.002	0.995–1.010
LRG1 0	0.100	0.018	29.765	< 0.001	1.105	1.066–1.145
Constant	–6.548	1.194	30.076	< 0.001	0.001	–

B represents the estimated regression coefficients for the covariates, with standard error (S.E.) given, OR represents ODDs ratio. The Wald statistics is the ratio of B to S.E. of the regression coefficient squared.

The binary logistic regression model was estimated using the maximum likelihood estimation (MLE). 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 assess 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 meant that the model fit the data well to a statistically acceptable level. Consequently, the model was able to correctly predict 92.8 % of those with appendicitis (1) and 89.3 % of those without appendicitis (0). Overall, 91.5 % of all cases (0.1) were correctly predicted. Another test statistic, the

Nagelkerke R2, was used to measure the usefulness of the model which indicates how useful the explanatory variables were in predicting the response variable. The Nagelkerke R2, which varies between 0 and 1, was 0.769, indicating that the model was useful in predicting appendicitis (Table 3.6). The logistic regression coefficient, standard error, Wald's chi-square, p-value, and odds ratio for each of the predictors are shown in Table 3.4. The Wald and associated p-value are used to test the statistical significance of each coefficient (B) in the model.

Table 3.6

Overall statistics of the Binary logistic regression models

Model	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square	Hosmer Lemeshow Chi-square
AA vs Ctr	74.535	0.562	0.769	5.518
AcA vs AuA	116.895	0.161	0.216	14.696

Model 2 was also statistically significant overall: model 2 (3.97) = 17.070 with a p-value of 0.001. The Hosmer-Lemeshow test statistic was not statistically significant ($X^2 = 14.696$, $p = 0.065$), indicating that the model fit the data well at a statistically acceptable level. Consequently, the model was able to correctly predict 65.4 % of those with complicated appendicitis (1) and 77.8 % of those with uncomplicated appendicitis (0). In total, 71.1 % of all cases (0.1) were correctly predicted. The Nagelkerke R2 was 0.216 indicating that the model was useful in predicting complicated appendicitis (Table 3.6). The logistic regression coefficient, standard error, Wald's chi-square, p-value, and odds ratio for each of the predictors are shown in Table 3.7.

Table 3.7

Binary logistic regression results and coefficient values used for this study

AcA vs AuA

Covariates	B	S.E.	Wald	p-value	OR	95 % CI for OR
IL-6 0	0.002	0.001	1.475	0.225	1.002	0.999–1.004
NGAL 0	0.003	0.002	1.608	0.205	1.003	0.998–1.008
LRG1 0	0.020	0.010	3.490	0.062	1.020	0.999–1.041
Constant	–2.149	0.870	6.101	0.014	0.117	–

B represents the estimated regression coefficients for the covariates, with standard error (S.E.) given, OR represents ODDs ratio. The Wald statistics is the ratio of B to S.E. of the regression coefficient squared.

The combined diagnostic model of IL-6, LRG1, NGAL in serum was established by binary logistic regression analysis (Table 3.8). The ROC curve showed that the 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

the 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).

Table 3.8

ROC

Model	AUC	95 % CI	p-value	Cut-off	Sensitivity	1-specificity	Specificity
AA vs Ctr	0.96	0.93–0.99	< 0.001	0.50	0.928	0.107	0.893
AcA vs AuA	0.74	0.63–0.84	< 0.001	0.49	0.673	0.222	0.778

3.2 Results – microbiota and susceptibility

Esherichia coli was the predominant representative of intraluminal appendiceal microbiota in both complicated and uncomplicated cases, in a total of 79 patients (81.4 %). *Pseudomonas aeruginosa* was the predominant microorganism of the extraluminal appendiceal microbiota (AcA/AuA: 15/5). There was no statistically significant difference between the results of the samples taken from the anatomical parts of the appendiceal lumen. 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 %) had different microbiota in the distal and proximal parts. In the AuA group, 24 cases (53 %) had identical microbiota, but in 21 cases (47 %) had different microbiota.

E. coli was the predominant species, with *P. aeruginosa* being the second most commonly isolated microorganism (Table 3.9). More than half of the patients (77.5 %), who received a drainage tube were diagnosed with AcA (p < 0.001). A comparison between the two groups suggests the median postoperative hospital stay was slightly shorter in the AuA group, five days versus six days. Preoperative *Y. enterocolitica* antibody detection was negative in all cases. Other demographic and clinical characteristics of the patients are shown in Table 3.10.

Table 3.9

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

Bacteria Type	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*
Bacterioides fragilis	2	40	3	60	5	0.665[*]

Table 3.9 continued

Bacteria Type	AcA		AuA		Total Isolates, No.	p-value
	No.	%	No.	%		
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.

Bacterial culture resulted in positive intraluminal samples with the growth of one or more strains from each appendix. Table 3.9 shows the number of cases of the most common isolates per the subdivision of AcA and AuA. Mixed strains were often found in culture. The most common bacteria isolated from the appendix were *E. coli* in 79, followed by *P. aeruginosa* in 20, *Klebsiella pneumoniae* in 6, *Bacterioides fragilis* in 5, and *Citrobacter braakii* in 5 samples (Table 3.9, Figure 3.14).

Table 3.10

Characteristics and distribution of study population

Indicator	AcA	AuA	Total	p-value
Children, n (%)	52 (53.6)	45 (46.4)	97	0.477
Age, median (IQR)	12 (9–14)	13 (10–15)	–	0.085
Laboratory Values, median (IQR)				
WBC count (x10^9/L),	16.91 (13.68–20.13)	14.64 (12.91–16.72)	–	**0.017**
CRP (g/L),	25.65 (5.28–87.22)	13.16 (2.96–38.57)	–	0.186
Neu	83.90 (80.40–87.00)	80.70 (73.95–84.75)	–	**0.028**
Alvarado Score, points, median (IQR)	8 (7–9)	7 (6–9)	–	0.092
Type of surgery, n (%)				
Laparotomy	8 (66.7)	4 (33.3)	12	
Laparoscopy	44 (51.8)	41 (48.2)	85	0.333
Drainage tube, n (%)	31 (77.5)	9 (22.5)	40	< **0.001**
Length of Hospital Stay, days, median (IQR)	6 (4–9)	5 (4–6)	–	0.002

AcA – Acute complicated appendicitis, AuA – Acute uncomplicated appendicitis, WBC – White Blood Cells, CRP – C–Reactive Protein., Median values are presented with IQR (25 %, 75 %)

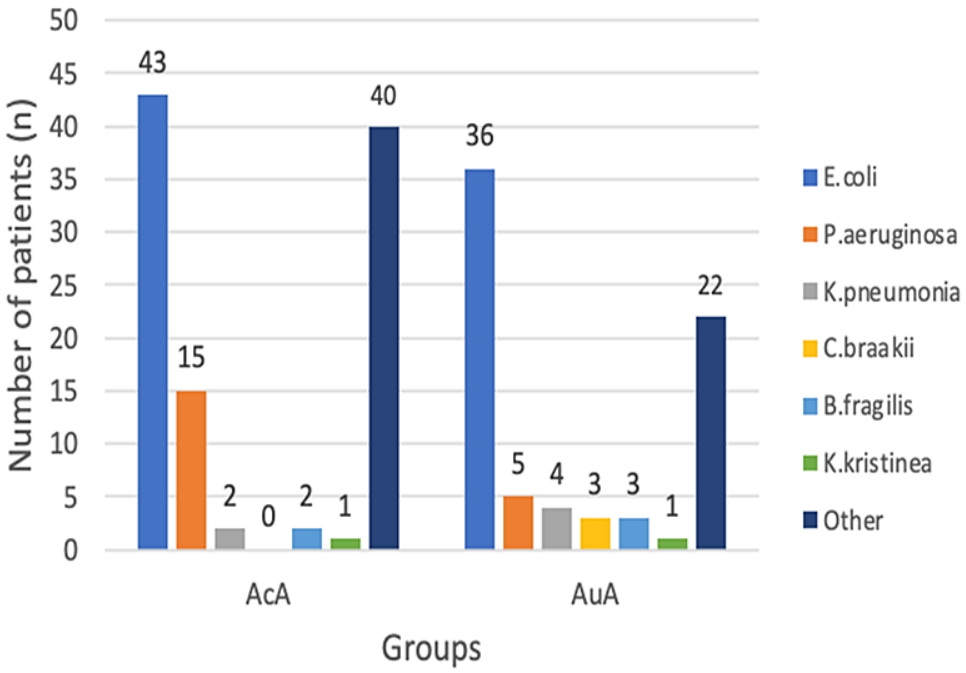


Figure 3.14 **Types of organisms isolated**

The 79 samples isolating *E. coli* showed various antibacterial susceptibilities, for example, 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. In addition, five ESBL-producing *E. coli* strains were also isolated.

P. aeruginosa, the second most common causative agent, showed a high prevalence in cases of acute complicated appendicitis. Susceptibility testing showed a good response to ceftazidime with only 26.3 % of isolates being resistant. Ampicillin resistance was found 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 strains tested were susceptible to meropenem, amikacin and gentamicin. The antibacterial susceptibility of other bacteria that were isolated in this study is shown in Table 3.11. *Citrobacter spp*. tested were resistant to all antibiotics except for amoxicillin/clavulanic acid, while *Klebsiella spp.* were resistant to cefotaxime, amikacin, gentamicin and chloramphenicol.

Table 3.11

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.

4 Discussion

Appendicitis remains difficult to diagnose and treat effectively in paediatric patients. This study focused on several elements – biomarkers, pathogens, and disease severity – to understand their correlations.

The enrolled patients were intentionally divided into AcA and AuA study groups based on intra-abdominal bacteriological findings and micro- and macro-perforation of the appendix *vermiformis*, rather than histological findings, taking into account the microbiological aspect of the study.

Research has shown that appendiceal luminal obstruction by the formation of a closed loop is the cause of appendicitis. Lymphoid hyperplasia in the follicles of the submucosa is the typical cause of luminal obstruction in children. Faecolith is mentioned as a precipitating obstructive factor, while parasites (e.g. nematodes) and inflammatory constrictions are less common causes (Bhangu et al., 2015). Obstruction leads to increased bacterial proliferation, thus increasing the intraluminal pressure, which subsequently impedes blood flow, leading to congestion and ischaemia, which promotes bacterial colonisation.

Although obstruction is the leading theory of the pathogenesis of appendicitis, it is not fully consistent with the data obtained in research and clinical practice. Therefore, bacteria are also thought to play a role in the pathogenesis. Another hypothesis stresses the importance of genetic predisposition as the prevalence of appendicitis is higher in first-degree relatives. Finally, perforated and non-perforated appendicitis representing the progression of the disease from early to late stages, are epidemiologically recognised as two distinct processes (Bhangu et al., 2015); (Abdurrazzaaq et al., 2018); (Essenmacher et al., 2018). There is a dispute regarding the most common causative agents being *Escherichia coli* and anaerobic *Clostridium perfringens*, while other authors cite *Klebsiella spp.* and *Enterobacter spp.*, or *Bacteroides fragilis*, *Pseudomonas aeruginosa* and *Peptostreptococcus spp*. as the most common (Parthiban, Harish, 2017); (Abdurrazzaaq et al., 2018); (Martin et al., 2023).

Choosing of the correct empirical antibacterial therapy is complex, as it requires a clinician to decide on the most appropriate antibiotic treatment prior to receiving the results of laboratory tests, isolating of the pathogen and determining its antimicrobial susceptibility. Therefore, in order to establish an accurate algorithm for the most effective empirical treatment, it is essential to know the most common pathogens in a given geographical region, their antimicrobial resistance profile and their ability to develop resistance to the most commonly used antibiotics (European Committee on Antimicrobial Susceptibility Testing (EUCAST): Clinical Breakpoints and Dosing of Antibiotics). There is some debate about the prevalent

causative agents, with some authors considering *E. coli* and anaerobic *Clostridium perfringens* to be the most common (Rekomendācijas Antibakteriālo Līdzekļu Lietošanai Ķirurģiskajā Praksē. KS/MET-011-00. Bērnu Klīniskā Universitātes Slimnīca. 2019) while others indicate *Klebsiella spp.* and *Enterobacter spp.* (Drugbank Online Ceftazidime.); or *Bacteroides fragilis*, *P. aeruginosa*, *Enterococcus spp*., alpha and gamma haemolytic streptococci as the most common (EUCAST: Clinical Breakpoints and Dosing of Antibiotics).

P. aeruginosa was one of the common causative agents isolated in our study, however, the spectrum of ceftriaxone does not cover this microorganism; therefore, cefotaxime would also not be an appropriate choice for the treatment of acute complex appendicitis (Nguyen et al., 2018). The results of our research show that approximately 53 % of *P. aeruginosa* isolates were resistant to cefotaxime, and about a third against ceftazidime. Cefotaxime is included in the Children's Clinical University Hospital guidelines for treatment efficacy of up to 90 % against ceftazidime-resistant strains of *P. aeruginosa* (Mazuski et al., 2016); (Qin et al., 2017).

Although both cefotaxime and ceftazidime are third-generation cephalosporins, ceftazidime-resistant strains have been identified. According to research data, the frequency of resistance to ceftazidime in *P. aeruginosa* isolates in Eastern European countries is approximately 26 %, which is highly consistent with the results of our study (Pilmis et al., 2021). Mechanisms of resistance to ceftazidime in *P. aeruginosa* include the production of beta-lactamase encoded by genes acquired by horizontal gene transfer or by increased production of a drug-induced, broad-spectrum, chromosomally encoded class C beta-lactamase with altered affinity (University of California San Francisco. Pediatric Appendicitis Clinical Algorithm, 2019). Intrinsic antimicrobial resistance of *P. aeruginosa* must also be taken into account. The purpose of using ceftazidime for the non-surgical treatment of simple appendicitis is to limit bacterial growth associated with *P. aeruginosa* within the appendix, to prevent the destruction of the appendiceal wall and its subsequent perforation. Further research is needed to determine the practical implications of our findings.

In our study, *P. aeruginosa* was prevalent in samples obtained from patients with acute complicated appendicitis. All strains were sensitive to meropenem, which inhibits cell wall synthesis and is not affected by beta-lactamase (Sarkar et al., 2017). Drusano et al. 2018 investigated the potential use of fosfomycin in the treatment of *P. aeruginosa* infections and found that the bacteria rapidly developed resistance to fosfomycin. Therefore, they suggested switching treatment from monotherapy to combination therapy with fosfomycin and meropenem. A synergistic effect was observed with fosfomycin wiping out the meropenem-resistant mutants and meropenem working against the fosfomycin-resistant strains. As a result, this combination was recommended as a treatment strategy for wider use in the future.

Another combination showing encouraging results in research settings is meropenem in combination with ceftazidime (Schulin et al., 2017). Over the past decade, antibiotic combinations of ceftazidime/avibactam, ceftolozane/tazobactam and piperacillin/tazobactam have been investigated as potential treatment options (Fournier et al., 2021).

Avibactam is a member of the class of azabicycloalkanes. Avibactam is a non-beta-lactam beta-lactamase inhibitor available in combination with ceftazidime. This combination was approved by the Food and Drug Administration (FDA) on 25 February 2015 for the treatment of complicated intra-abdominal infections in combination with metronidazole (Drusano et al., 2018). This combination has shown an efficacy of up to 90 % against ceftazidime-resistant strains of *P. aeruginosa* (Feng et al., 2017). Combined treatment with ceftazidime-avibactam and colistin has shown promise in the treatment of XDR (extremely drug-resistant) *P. aeruginosa* infections (Naka, Fujimoto, 2018). Ceftazidime and avibactam cannot be used against microorganisms with intrinsic resistance. Strains resistant to ceftazidime and avibactam should be treated with other effective antimicrobials or in combination with other antibiotics (Drusano et al., 2018).

Ceftolozane-Tazobactam. Ceftolozane-tazobactam was approved by the FDA in 2014, shortly before ceftazidime-avibactam was approved for the same indications. It is highly effective in combinations with meropenem and levofloxacin (Solomkin et al., 2015); (Wagenlehner et al., 2015); (Nguyen et al., 2018).

Piperacillin/Tazobactam. The combination of piperacillin/tazobactam includes an anti-pseudomonal penicillin and a beta-lactamase inhibitor. The mechanism of action is based on inhibiting the biosynthesis of cell wall mucopeptides by binding to one or more penicillin-binding proteins. The antibiotic is highly effective during the growth or log phase (Anonymous, 2021). Treatment protocols vary widely, but most commonly involve hospital treatment for one to two days (e.g. piperacillin/tazobactam, ceftriaxone and metronidazole or ciprofloxacin and metronidazole) until symptoms resolve and the WBC count normalises. This is followed by outpatient oral antibiotic therapy (e.g. amoxicillin/clavulanic acid or ciprofloxacin and metronidazole) (Georgiou et al., 2017).

In our research, amikacin showed significant efficacy against isolates from the samples. It is a broad-spectrum semi-synthetic aminoglycoside antibiotic, derived from kanamycin with antimicrobial properties. Amikacin binds irreversibly to the bacterial 30S ribosomal subunit, specifically trapping 16S rRNA and S12 protein within the 30S subunit. This leads to interference with the translation initiation complex and misreading of mRNA, preventing protein synthesis, and resulting in the bactericidal effect. This agent is typically used for short-term treatment of severe infections caused by susceptible strains of gram-negative bacteria

(Anonymous, 2019). Data on amikacin-resistant Pseudomonas are scarce. Research conducted by Loho et al. showed that only two of the 20 *P. aeruginosa* isolates were resistant to amikacin. Its combination with doripenem is synergistic and improves treatment results (Loho et al., 2018).

The most isolated microorganism from our patients' samples was *E. coli*, especially those treated for acute uncomplicated appendicitis. This finding concurs with the results obtained by other authors (Bhangu et al., 2015); (Abdurrazzaaq et al., 2018); (Bazzaz et al., 2018); (Essenmacher et al., 2018); (Rickard et al., 2018); (Snyder et al., 2018); (Turel et al., 2019). Our data reveal that strains of *E. coli* are sensitive to antibacterial agents such as amikacin, imipenem, and meropenem, which is in line with recent studies by other researchers (Hao et al., 2016). There were strains resistant to other antibacterial agents included in the treatment guidelines, such as cefotaxime and ceftazidime were six and five out of 59, respectively. Only five isolates (8,5 %) were ESBL-positive. This is consistent with data from other studies determining the prevalence of ESBL-producing *E. coli* in Latvia. 11 % of *E. coli* present in animal microbiota produce ESBL, whereas in the adult population, ESBL are produced by only 1.6 % of E. coli (Kakar et al., 2020); (Kakar et al., 2021).

To prevent further spread of infection in cases of acute appendicitis with complications, such as perforation, empirical treatment could include ceftriaxone (in combination with metronidazole) or ertapenem for children over one month of age. Other options for empirical treatment could include piperacillin/tazobactam, imipenem or meropenem. The main aim of an appropriate antibacterial treatment regimen is to prevent complications associated with the infection. Empirical antibiotic treatment should be based on information about the most isolated microorganisms in a given area and their antimicrobial resistance profile (Terentjeva et al., 2019).

Appendicitis remains a major diagnostic challenge, relying heavily on clinical assessment of patients and scoring systems to guide diagnosis. Modern diagnostic methods are used to confirm the diagnosis of appendicitis and to predict its course and severity (Podany et al., 2017). Current inflammatory markers such as leucocytosis and CRP are too vague to accurately diagnose appendicitis with high specificity or sensitivity (Almaramhy et al., 2017). The success of an efficient diagnostic scheme provided by inflammatory biomarkers can drastically reduce healthcare costs, increase the accuracy and speed of diagnosis, and provide non-invasive treatments, In recent years, numerous attempts to find a single biomarker that would be indicative of paediatric AA have yielded insufficient results, especially in differentiating AcA from AuA (Daly et al., 2017); (Kakar et al., 2020); (Rogers et al., 2016).

For this reason, a combination of biomarkers is likely to be the most accurate way to make the correct diagnostic and treatment decisions.

In this part of the study, we compared three biomarkers: LRG1, NGAL and IL-6 in the diagnosis of acute appendicitis in children and its severity. According to the results, S-NGAL, S-LRG1, U- LRG1 and S-IL-6 can be effective biomarkers in the differential diagnosis of acute abdominal pain in children presenting to the emergency department depending on the onset of symptoms.

The primary objective of this study was to evaluate these three biomarkers in differentiating acute complicated appendicitis from AuA. S-LRG1 concentrations were significantly elevated in the AcA group compared to the AuA group, and in AuA and AcA compared to Ctr. This indicates that S-LRG1 correlates with the severity of appendicitis and could be used in clinical life to predict high vulnerability to complicated appendicitis (sensitivity 59.6 % and specificity 77.8 %). This could be explained by neutrophils secreting LRG1 in the presence of bacteria (Naka, Fujimoto, 2018). Its role in inhibiting cell apoptosis by binding to cytochrome C stimulates lymphocyte survival in the appendix and protects appendiceal tissue from susceptibility to toxicity (Rainer et al., 2017). In addition, LRG1 binds to accessory receptors of transforming growth factor-b and regulates a signalling pathway that stimulates angiogenesis, which may enhance tissue inflammation (Hao et al., 2016). Neutrophils act as first responders to infection, which explains the rapid detection of AA by LRG1, as it is secreted by neutrophils. Additionally, LRG1 has a longer half-life than CRP, so the time range of AA is in favour of LRG1 (Naka, Fujimoto, 2018); (Wagatsuma et al., 2021). However, Rainer et al. 2017 showed that both whole-blood LRG1 mRNA and plasma LRG1 concentrations were elevated in patients with AA, which may have a role as a diagnostic marker.

The difference in serum IL-6 was significant in all three groups. IL-6 alone has been shown to have sensitivity of 67.3 % and specificity of 71.1 % in differentiating between AcA and AuA (Kakar et al., 2021). IL-6 polymorphisms have previously been associated with the severity of AA. In our study, serum IL-6 concentrations were significantly elevated in the AcA group compared to the AuA group. Peeters et al., 2020, have also found that IL-6, interleukin-8 (IL-8) and tumour necrosis factor alpha (TNF alpha) levels were higher in complicated appendicitis.

Our previous study in a smaller cohort suggested that the levels of NGAL levels were elevated preoperatively in paediatric patients with acute appendicitis and low in patients without abdominal inflammation, but not strong enough to differentiate between AcA and AuA (Kakar et al., 2020). Bakal et al., 2016, have concluded that NGAL is useful for the diagnosis of

paediatric appendicitis, and our results support this finding. NGAL is expressed in neutrophils and at low levels in the renal system, and gastrointestinal and respiratory tracts. When one or more of these organ tissues suffer epithelial damage, the serum level of NGAL temporarily increases. NGAL is secreted into the blood at high levels within two hours of injury (Kari et al., 2018). According to this study, NGAL can be used as a marker, preferably together with LRG1 and IL-6 to determine between AcA and AuA. When these biomarkers are used together, the diagnostic accuracy, sensitivity and specificity reach higher levels. In conclusion, NGAL could be used as a predictive biomarker in the early stages or to establish the diagnosis, however, its level decreases within a few days. Therefore, the time frame for using NGAL requires further research to evaluate when the biomarker has greater diagnostic value.

Our previous study concluded that LRG1 was significantly higher in the AA group compared to the control group and that there is high sensitivity (93.8 %) and specificity (91.1 %) for diagnosing AA with S-LRG1. The cut-off value proposed is 51.69 μg/ml (Kakar et al., 2021). Yap et al. suggested that S-LRG1 analysis could replace the IL-6 value in the Alvarado score. This could help to diagnose AA without radiological confirmation, which is supported by this study. According to the results of this study, the S-LRG1 ROC curve shows greater accuracy and is therefore a better choice than IL-6 for the diagnosis of AA. Subacute appendicitis cannot be detected by CRP, IL-6, or leukocytes.

Non-invasive diagnostic methods, especially for children, are more useful in clinical practice and were therefore included in the study. In this study, U-IL-6 and U-NGAL showed no significant difference between any of the groups, which was also seen in our previous study with a smaller study population. Conditions with kidney injury can increase U-NGAL, as proven by Kari et al. 2018 in relation to kidney injury. Since there is no renal damage in appendicitis, this presumably leads to the normal levels of NGAL in urine (Kari et al., 2018). In contrast, U-LRG1 levels are significantly elevated in paediatric appendicitis, making it an excellent marker for paediatric appendicitis. It has been previously stated that LRG1 is an accurate diagnostic method, superior to current urinary inflammatory markers (Kentsis et al., 2012); (Naka, Fujimoto, 2018); (Kakar et al., 2021).

It should be emphasised that the results of the study in Latvia could improve the quality of medical care in countries with relatively low socio-economic status.

In a recently published systemic review and meta-analysis on leucine-rich alpha-2 glycoprotein 1 as a biomarker, the authors highlight LRG1 as a potential non-invasive biomarker for the diagnosis of paediatric acute appendicitis (Montero et al., 2023).

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 on Day 0 and should be used in the differential diagnosis of acute abdominal pain.
3. CRP and serum IL-6 remain as unspecific biomarkers and 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.

Limiting factors

There were some limitations to this study. It was a single-centre study with a small sample size. Shortage of resources during odd hours, courier services, microbiology laboratory working hours, restrictions due to the COVID-19 pandemic limited the number of participants. Most patients received either antibiotic and/or intravenous fluid therapy prior to surgery and sample collection, which may have affected biomarker concentrations. The use of ELISA kits in other publications makes comparison with the results of this study difficult. The analysis of samples was performed in a clinical research laboratory, not in the hospital laboratory, which may have affected concentrations. Patient quotas limited the range of patient types that could be included, but this study has provided a basis for future, broader studies that are already underway. Patients under seven years of age were excluded - as this age group tends to have a different pathophysiology for appendicitis, as there is a strong association with bouts of viral infections such as gastroenteritis - and pre-hospital history information can be quite limited. The diagnosis of appendicitis must be ruled out from non-specific abdominal pain, and laboratory investigations and surgical consultation are often the tools of the trade. Patients with non-surgical abdominal pain were not included in this study due to the patient quotas. It should be noted that biomarkers may have different levels within the time frame of pathology and therefore, non-specific results between patients presenting to the emergency department. Furthermore, the enrolled patients were intentionally divided into the AcA and AuA study groups based on intra-abdominal bacteriological findings and micro- and macro-perforation of the appendix vermiformis, rather than histological findings, taking into account the microbiological aspect of the study. Kari et al., 2018, suggested that the diagnosis of acute kidney injury (AKI) is more accurate when the values are examined within a time frame of 12 hours after the onset of the injury.

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. 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. Method of prognosing risk of development of gangrenous appendicitis in children. (Patent No. 15613). Patent Office of the Republic of Latvia. http://databases.lrpv.gov.lv/databases/lv/patent/details/lvp2021000038/

Reports at international congresses and conferences

1. Viksne, A., Engelis, A., Kakar, M., Laizans, P., Petersons, A. 2014. Criteria for non-operative management of acute appendicitis in children. 13th Conference of the Baltic Association of Pediatric Surgeons. Abstracts. Vilnius, September, 25–27, 2014, 31
2. Zviedre, A., Engelis, A., Tretjakovs, P., Kakar, M., Petersons, A. 2013. Serum level of cytokines allows differentiating acute appendicitis and acute mesenteric lymphadenitis in children. 14th EUPSA Congress. Abstracts. Leipzig, June 5–8, 2013, (PO247), 351.
3. Zviedre, A., Engelis, A., Tretjakovs, P., Kakar, M., Petersons, A. 2012. Significance of cytokines in acute appendicitis and acute mesenteric lymphadenitis in children. 13th EUPSA Congress and 59th BAPS Congress. Abstracts. Rome, June 13–16, 2012, (PO267), 263
4. Zviedre, A., Kakar, M., Engelis, A., Petersons, A. 2014. Alvorado scoring system, ultrasound, and C - reactive protein in diagnosis of acute appendicitis. Abstract Book – The 15th European Congress of Paediatric Surgery. Dublin, Ireland, June 18–21, 2014, 278.
5. de Beaufort CMC et al., Transitional Care for Patients with Congenital Colorectal Diseases: An EUPSA Network Office, ERNICA, and eUROGEN Joint Venture, *Journal of pediatric surgery*, https://doi.org/10.1016/j.jpedsurg.2023.06.008
6. Engelis, A., Smane, L., Pavare, J., Zviedre, A., Zurmutai, T., Berezovska, M.,M., Bormotovs, J., Kakar, M., Saxena, A. K., Petersons, A. 2021. Case Series of Variable Acute Appendicitis in Children with SARS-CoV-2 Infection. Children 2021, 8, 1207. https://doi.org/10.3390/children8121207
7. Franckevica, I., Lubgane, M., Kakar, M., Zviedre, A. 2015. Granulamatous appendicitis as a rare cause of appendectomy in childhood. 8th Congress of Baltic Association of Surgeons. Abstracts. Tallinn, September, 10–12, 2015, 27.

8. Kakar, M., Broks, R., Asare, L., Delorme, M., Berezovska, M. M., Zviedre, A., Reinis, A., Eņģelis, A., Kroiča, J., Saxena, A. K., & Pētersons, A. 2021. Biomarkers Leucine Rich Alpha-2 Glycoprotein 1, Neutrophil Gelatinase- Associated Lipocal and Interleukin-6 Diagnose Pediatric Acute Appendicitis. Poster session presented at 22nd Annual Congress of the European Paediatric Surgeons' Association (EUPSA), Athens, Greece.

9. 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. 33rd International Symposium on Pediatric Surgical Research. Abstracts.- Frankfurt (Main), Germany, November, 27–29, 2020.

10. Kakar, M., Eņģelis, A., Kroiča, J., Reinis, A., Broks, R., Asare, L., Zurmutaī, T., Zviedre, A., Saxena, A., & Pētersons, A. 2022. Role of multiple biomarkers in predicting the diagnosis and severity of acute appendicitis in children. 214. 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

11. Kakars, M., Engelis A., Kroica J., Saxena A. K., Reinis A., Butnere M.M., Viksne A., Petersons A. 2018. Complex clinical, biological and microbiological analysis of acute complicated and acute uncomplicated appendicitis in children. The 15th conference of the Baltic Association of Paediatric Surgeons. Riga, Latvia, May 10–12, 2018, 59.

12. Kakar, M., Gasiņa, L., Kroiča, J., Reinis, A., Broks, R., Asare, L., Zviedre, A., Zurmutaī, T., Ābola, Z., Eņģelis, A., Saxena, A., Pētersons, A. 2023. Cinical, molecular biological, and microbiological integrated investigation in the case of paediatric acute complicated and uncomplicated appendicitis. Riga Stradiņš University Research Week 2023. Abstracts.- Riga, Latvia, March, 29, 2023.

13. Liepina, Z., Tauriņa, L., Asare, L., Zviedre, A., Kakar, M., Eņģelis, A., & Pētersons, A. 2022. Diagnostic Criteria for the Treatment of Pediatric Acute Appendicitis Using Clinical Recomendations in Latvia. 266–266. 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

14. Kakars, M., Engelis, A., Rekevica, A., Soldatenkova, K., Laizans, P., Petersons, A. 2016. Acute appendicitis in children: a single centre overview of epidemiology and diagnosis (2010–2015). 14th Conference of the Baltic Association of Pediatric Surgeons. Abstracts.- Tartu, May, 5–8, 2016, 26.

15. Kakars, M., Engelis, A., Petersons, A., Titans, V., Zviedre, A. 2016. Management overview of acute appendicitis in children: a single-centre experience (2000-2015). 5th World congress of Pediatric Surgery. Abstracts. Washington, DC, United States, October, 8–11, 2016, 100-PA

16. Kakar, M., Kroica, J., Reinis, A., Broks, R., Asare, L., Smane, L., Senica, S. O., Vermeulen, M., Zviedre, A., Zurmutai, T., Laizans, P., Abola, Z., Engelis, A., Saxena, A., Petersons, A. 2022. Diagnostic Accuracy Of Multiple Biomarkers In Predicting The Severity Of Acute Pediatric Appendicitis In The Emergency Department. 23rd European Paediatric Surgeons' Association (EUPSA 2022) Congress. Abstract. Tel Aviv, Israel, June, July, 29–2.

17. Kakar, M., Kroica, J., Reinis, A., Butnere, M, M., Broks, R., Asare, L., Zviedre, A., Zurmutai, T., Nagle, I., Gasina, L., Engelis, A., Saxena, A., Petersons, A. 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.

18. Kakar, M., Kroica, J., Reinis, A., Butnere, M, M., Broks, R., Asare, L., Zviedre, A., Zurmutai, T., Nagle, I., Gasina, L., Engelis, A., Saxena, A., Petersons, A. Microbiota diversity affects acute appendicitis severity in children. 2023. 16th Conference of the Baltic Association of Paediatric Surgeons. Abstract. Kaunas, Vilnius, September 14–16.

19. Kakars, M., Broks, R., Asare, L., Delmore, M., Brezovska, M. M., Zviedre, A., Reinis, A., Engelis, A., Kroica, J., Saxena, A., Petersons, A. 2019. 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). The 6th World Congress of the World Federation of Associations of Pediatric Surgery (WOFAPS 2019). Abstracts. – Doha, Qatar, November, 1–3, 2019.
20. Soyer, T., Dariel, A., Dingemann, J., Martinez, L., Pini-Prato, A., Morini, F., De Coppi P., Gorter, R., Doi, T., Sindjic Antunovic, S., Kakar M., Hall N. J. 2022. European Pediatric Surgeons' Association Survery on the Management of Primary Spontaneous Pneumothorax in Children. European *Journal of pediatric surgery*. January 2022. https://doi.org/10.1055/s-0041-1739420.
21. Van Amstel, P., El Ghazzaoui, A., Hall, N.,J., Wester, T., Morini, F., van der Lee, J.H., Singer, G., Pierro, A., Zani, A., Gorter, R., and the CONNECT collaborative study group. 2022. Paediatric appendicitis: international study of management in the COVID-19 pandemic. BJS, 1–5.
22. Viksne, A., Abola, Z., Engelis, A., Butnere, M.,M., Kakar, M., Zviedre, A., Petersons, A. 2017. Feasibility and safety of cost effective antimicrobial therapy for nonsurgical management of acute appendicitis in children. The 18th European Congress of Paediatric Surgery. Limassol, Cyprus, June 17–20, 2017, 58.

Reports at local congresses and conferences

1. An update of a single center review in the management of acute appendicitis in children: 2000–2016. (Mohits Kakars, Agnese Rekevica, Arnis Engelis, Aigars Petersons). Rīgas Stradiņa universitāte. 2017. gada medicīnas nozares zinātniskās konferences tēzes, 59.
2. Kakars, M., Broks, R., Asare, L., Delmore, M., Berezovska, M. M., Zviedre, A., Reinis, A., Kroiča, J., Eņģelis, A., Saxena, A. K., & Pētersons, A. 2021. Determining acute complicated and uncomplicated appendicitis using serum and urine biomarkers leucine rich alpha-2 glycoprotein 1, neutrophil gelatinase-associated lipocal and interleukin-6. 52. Abstract from RSU Research week 2021: Knowledge for Use in Practice, Rīga, Latvia.
3. Kakars, M., Broks, R., Reinis, A., Saxena, A., Eņģelis, A., Kroiča, J., & Pētersons, A. 2020. Evaluation of appendical microbiota in children with acute complicated and UN complicated appendicitis. 31. Abstract from IX Latvian Gastroenterology Congress, Riga, Latvia. https://www.gastroenterologs.lv/content_graphics/user_file/pdf/Gastroenterologu_kongress_Abstracts_ENG_WEB.pdf
4. Kakars, M., Kroiča, J., Reinis, A., Broks, R., Asare, L., Delmore, M., Berezovska, M. M., Zviedre, A., Eņģelis, A., Saxena, A. K., & Pētersons, A. 2021. Evaluation of microbiota in children with acute appendicitis. 271. Abstract from RSU Research week 2021: Knowledge for Use in Practice, Rīga, Latvia.
5. Evaluation of microbiota in children with acute appendicitis. Oral presentation – RSU Research week 2021: Knowledge for Use in Practice. Riga, Latvia, March 24, 2021.

Scientific articles in peer-reviewed publications published in Latvia

1. Pētersons, A., Enģelis, A., Zviedre, A., Ābola, Z., Kakars. M. 2010. Biežākās akūtās vēdera dobuma ķirurģiskās slimības bērniem. *ĀRSTS.* Nr. 2, 28–30.
2. Eņģelis, A., Kakar, M., Zviedre, A., Laizāns, P., Zurmutai, T., Bormotovs, J., Pētersons, A. 2022. Surgical and non-surgical treatment of paediatric appendicitis: can algorithms help us to predict perforation? *Proceedings of the Latvian Academy of Sciences.* Section B. Vol. 76, No. 5/6 (740/741), 595–601.
3. Zviedre, A., Engelis, A., Kakar, M., Petersons A. 2011. Potential Role of Cytokines in Children with Acute Appendicitis and Acute Mesenteric Lymphadenitis. *Acta Chirurgica Latviensis*. Nr.11, 130–133 (Published in Versita, CAB Abstracts Global Health).

4. Zviedre, A. Eņģelis, A., Tretjakovs, P., Pētersons, A., Kakars, M. 2012. Citokīnu grupas seruma iekaisuma mediatoru (CGSIM) dinamiskās svārstības un to nozīme bērniem ar akūtu apendicītu (AAp) un akūtu mezenteriālu limfadenītu (AMLa). *RSU Zinātniskie raksti*. Rīga: RSU, 38–44.

Bibliography

1. Abdurrazzaaq, A., Afuwape, O., Ademola, A., & Fasina, O. 2018. Bacterial pattern in acute appendicitis. *Annals of African Surgery*, 15(1). https://doi.org/10.4314/aas.v15i1.3
2. Aiyoshi, T., Masumoto, K., Tanaka, N., Sasaki, T., Chiba, F., Ono, K., Jimbo, T., Urita, Y., Shinkai, T., Takayasu, H., et al. 2021. Optimal First-Line Antibiotic Treatment for Pediatric Complicated Appendicitis Based on Peritoneal Fluid Culture. *Pediatr. Gastroenterol. Hepatol. Nutr.*, 24, 510–517. http://doi.org/10.5223/pghn.2021.24.6.510
3. Almaramhy, H. H. 2017. Acute appendicitis in young children less than 5 years: review article. *Ital J Pediatr*, 43, 15. https://doi.org/10.1186/s13052-017-0335-2
4. Anonymous. 2019. National Center for Biotechnology Information. PubChem Database. Amikacin, CID=37768. https://pubchem.ncbi.nlm.nih.gov/compound/Amikacin (last accessed December 12, 2019).
5. Anonymous. 2021. Piperacillin/tazobactam (Rx). https://reference.medscape.com/drug/zosyn-piperacillin-tazobactam-342485#10 (last accessed on 7 July, 2021).
6. Arredondo Montero, J., Pérez Riveros, B. P., Bueso Asfura, O. E. et al. 2023. Leucine-Rich Alpha-2-Glycoprotein as a non-invasive biomarker for pediatric acute appendicitis: a systematic review and meta-analysis. *Eur J Pediatr*. https://doi.org/10.1007/s00431-023-04978-2
7. Bakal, U., Saraç, M., Ciftci, H., Tartar, T., Kocdemir, E., Aydin, S., Kazez, A. 2016. Neutrophil gelatinase-associated lipocal in protein levels as an acute appendicitis biomarker in children. *SpringerPlus* 5(1):1–5. https ://doi.org/10.1186/s4006 4-016-1853-x
8. Bazzaz, A. A., Lor, D. A. K., & Mahdi, N. B. 2018. Impact of some antibiotics on bacteria isolated from appendices in Kirkuk Province, Iraq. *Advances in Bioscience and Biotechnology*, 09(01), 1–10. https://doi.org/10.4236/abb.2018.91001
9. Becker, P., Fichtner-Feigl, S., & Schilling, D. 2018. Clinical Management of Appendicitis. *Visceral medicine*, 34(6), 453–458. https://doi.org/10.1159/000494883
10. Bhangu, A., Søreide, K., Di Saverio, S., Assarsson, J. H., Drake, F. T. 2015. Acute appendicitis: modern understanding of pathogenesis, diagnosis, and management. *Lancet*. 26;386(10000):1278–1287. doi: 10.1016/S0140-6736(15)00275-5.
11. Bhattacharya, J., Silver, E. J., Blumfield, E., Jan, D. M., Herold, B. C., Goldman, D. L. 2022. Clinical, Laboratory and Radiographic Features Associated with Prolonged Hospitalization in Children with Complicated Appendicitis. *Front Pediatr*.; 10:828748. doi: 10.3389/fped.2022.828748.
12. Camacho-Cruz, J., Padilla, P. O., Sánchez, D.G. et al. 2022. Outcomes of acute appendicitis in patients younger than age 4: a descriptive study. *Ann Pediatr Surg* 18, 59. https://doi.org/10.1186/s43159-022-00196-x
13. Camilli, C., Hoeh, A. E., De Rossi, G. et al. 2022. LRG1: an emerging player in disease pathogenesis. *J Biomed Sci* 29, 6. https://doi.org/10.1186/s12929-022-00790-6
14. Chen, C. Y., Chen, Y. C., Pu, H. N., Tsai, C. H., Chen, W. T., Lin, C. H. 2012. Bacteriology of acute appendicitis and its implication for the use of prophylactic antibiotics. *Surg Infect (Larchmt)*;13(6):383–90. doi: 10.1089/sur.2011.135.
15. Coccolini, F., Fugazzola, P., Sartelli, M., Cicuttin, E., Sibilla, M. G., Leandro, G., De' Angelis, G. L., Gaiani, F., Di Mario, F., Tomasoni, M., Catena, F., & Ansaloni, L. 2018. Conservative treatment of acute appendicitis. *Acta bio-medica: Atenei Parmensis*, 89(9-S), 119–134. https://doi.org/10.23750/abm.v89i9-S.7905
16. Daly, M. C., von Allmen D, Wong, H. R. 2018. Biomarkers to estimate the probability of complicated appendicitis. *J Pediatr. Surg*.; 53(3):437–440. doi: 10.1016/j.jpedsurg.2017.09.004.
17. Drugbank Online. Ceftazidime. Available online: https://go.drugbank.com/drugs/DB00438 (accessed on 6th of June 2022).

18. Drusano, G. L., Neely, M. N., Yamada, W.M., Duncanson, B., Brown, D., Maynard, M., Vicchiarelli, M., Louie, A. 2018. The combination of fosfomycin plus meropenem is synergistic for Pseudomonas aeruginosa PAO1 in a hollow-fiber infection model. *Antimicrob Agents Chemother*., 62, e01682-18. https://doi.org/10.1128/AAC.01682-18

19. Essenmacher, A. C, Nash, E., Walker, S. K, Pitcher, G. J., Buresh, C. T, & Sato, T. S. 2018. Stump Appendicitis. Clinical Practice and Cases in Emergency Medicine, 2(3). http://dx.doi.org/10.5811/cpcem.2018.3.3773

20. European Committee on Antimicrobial Susceptibility Testing. Clinical Breakpoints and Dosing of Antibiotics. Available online: http://www.eucast.org/clinical_breakpoints/ (accessed on 10th of December 2020)

21. Feng, Y., Bakker, R. T., van Hest, R. M., Hodiamont, C. J., Brul, S., Schultsz, C., ter Kuile, B. H. 2017. Optimization of therapy against Pseudomonas aeruginosa with ceftazidime and meropenem using chemostats as model for infections. *FEMS Microbiol. Letters*, 364, fnx142. http://doi.org/10.1093/femsle/fnx142

22. Fernandes, S., Vasconcelos-Castro, S., Teixeira, C., & Soares-Oliveira, M. 2020. Yersinia Enterocolitis May Mimic Appendicitis: 12 Years of Experience in a Single Tertiary Center. *GE Portuguese journal of gastroenterology*, 28(1), 26–31. https://doi.org/10.1159/000507555

23. Ferris, M., Quan, S., Kaplan, B. S., Molodecky, N., Ball, C. G., Chernoff, G. W., Bhala, N., Ghosh, S., Dixon, E., Ng, S., Kaplan, G. G. 2017. The Global Incidence of Appendicitis: A Systematic Review of Population-based Studies. *Ann Surg.*; 266(2):237–241. doi: 10.1097/SLA.0000000000002188.

24. Fournier, D., Carrière, R., Bour, M., Grisot, E., Triponney, P., Muller, C., Lemoine, J., Jeannot, K., Plésiat, P., the GERPA Study Group. 2021. Mechanisms of resistance to ceftolozane/tazobactam in Pseudomonas aeruginosa: Results of the GERPA multicenter study. *Antimicrob Agents Chemother*., 65, e01117-20. http://doi.org/10.1128/AAC.01117-20

25. Georgiou, R., Eaton, S., Stanton, M. P., Pierro, A., & Hall, N. J. 2017. Efficacy and safety of nonoperative treatment for acute appendicitis: A meta-analysis. *Pediatrics*, 139(3). https://doi.org/10.1542/peds.2016-3003

26. Guillet-Caruba, C., Cheikhelard, A., Guillet, M., Bille, E., Descamps, P., Yin, L., Khen-Dunlop, N., Zahar, J.-R., Sarnacki, S., Révillon, Y., Berche, P., & Ferroni, A. 2011. Bacteriologic epidemiology and empirical treatment of pediatric complicated appendicitis. *Diagnostic Microbiology and Infectious Disease*, 69(4), 376–381. https://doi.org/10.1016/j.diagmicrobio.2010.11.003

27. Hajibandeh, S., Hajibandeh, S., Marshall, M. J., Smart, N. J., Winyard, P. G., Hyde, C., Shaw, A. M., & Daniels, I. R. 2021. Biomarkers for diagnosis of acute appendicitis in adults. *The Cochrane Database of Systematic Reviews*, 2021(10), CD011592. https://doi.org/10.1002/14651858.CD011592.pub2

28. Hao, L., Xie, H., Zhang, B., Chen, D., Wang, S., Zhang, H., He, S. LRG1 downregulation in allergic airway disorders and its expression in peripheral blood and tissue cells. *J. Transl. Med*. 2016, 14, 202. http://doi.org/10.1186/s12967-016-0929-2

29. Heather F. Smith, William Parker, Sanet H. Kotzé, Michel Laurin. Morphological evolution of the mammalian cecum and cecal appendix. *Comptes Rendus Palevol*, 2017; 16 (1): 39 DOI: 10.1016/j.crpv.2016.06.001

30. Hodge, VL, Sarah,. Mickiewicz, Beata., Lau, Matthew., Jenne, N, Craig., Thompson, C, Graham. 2021. Novel molecular biomarkers and diagnosis of acute appendicitis in children. *Biomarkers in Medicine*, Vol 15. NO. 12. https://doi.org/10.2217/bmm-2021-0108

31. Hong Duc, P., Minh Xuan, N., Huu Thuyet, N., & Quang Huy, H. 2020. Intestinal Obstruction due to Acute Appendicitis. *Case reports in gastroenterology*, 14(2), 346–353. https://doi.org/10.1159/000508435

32. Hosseinpour, M., & Ahmadi, B. 2016. Emergency Abdominal Surgery in Infants and Children. *InTech*. doi: 10.5772/63649

33. Kakar M, Berezovska MM, Broks R, et al. Serum and Urine Biomarke Leucine-Rich Alpha-2 Glycoprotein 1 Differentiates Pediatric Acute Complicated and Uncomplicated Appendicitis. *Diagnostics*. 2021;11(5):860.

34. Kakar M, Delorme M, Broks R, et al. Determining acute complicated and uncomplicated appendicitis using serum and urine biomarkers: interleukin-6 and neutrophil gelatinase-associated lipocalin. *Pediatric Surgery International*. 2020;36(5):629-636.

35. Kari JA, Shalaby MA, Sofyani K et al 2018 Urinary neutrophil gelatinase-associated lipocalin (NGAL) and serum cystatin C measurements for early diagnosis of acute kidney injury in children admitted to PICU. *World J Pediatr* 14(2):134–142. https ://doi.org/10.1007/s1251 9-017-0110-x

36. Kentsis, A., Ahmed, S., Kurek, K., Brennan, E., Bradwin, G., Steen, H., & Bachur, R. 2012. Detection and diagnostic value of urine leucine-rich α-2-glycoprotein in children with suspected acute appendicitis. *Annals of emergency medicine*, 60(1), 78–83.e1. https://doi.org/10.1016/j.annemergmed.2011.12.015

37. Kentsis, A., Lin, Y. Y., Kurek, K., Calicchio, M., Wang, Y. Y., Monigatti, F., Campagne, F., Lee, R., Horwitz, B., Steen, H., & Bachur, R. 2010. Discovery and validation of urine markers of acute pediatric appendicitis using high-accuracy mass spectrometry. *Annals of emergency medicine*, 55(1), 62–70.e4. https://doi.org/10.1016/j.annemergmed.2009.04.020

38. Loho, T., Sukartini, N., Astrawinata, D. A. W., Immanuel, S., Aulia, D., & Priatni, I. 2018. In Vitro Antibacterial Interaction of Doripenem and Amikacin against Multidrug-Resistant Acinetobacter baumannii, Pseudomonas aeruginosa, and Klebsiella pneumoniae Isolates. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 1–6. https://doi.org/10.1155/2018/1047670

39. Maloney, C., Edelman, M. C., Bolognese, A. C., Lipskar, A. M., & Rich, B. S. 2019. The Impact of Pathological Criteria on Pediatric Negative Appendectomy Rate. *Journal of pediatric surgery*, 54(9), 1794–1799. https://doi.org/10.1016/j.jpedsurg.2018.10.106

40. Martin, B., Subramanian, T., Arul, S., Patel, M., & Jester, I. 2023. Using Microbiology Culture in Pediatric Appendicitis to Risk Stratify Patients: A Cohort Study. Surgical infections, 24(2), 183–189. https://doi.org/10.1089/sur.2022.220

41. Mazuski, J. E., Gasink, L. B., Armstrong, J., Broadhurst, H., Stone, G. G., Rank, D., Llorens, L., Newell, P., & Pachl, J. 2016. Efficacy and safety of ceftazidime-avibactam plus metronidazole versus meropenem in the treatment of complicated intra-abdominal infection: Results from a randomized, controlled, double-blind, phase 3 program. Clinical Infectious Diseases, 62(11), 1380–1389. https://doi.org/10.1093/cid/ciw133

42. Medscape. Piperacillin/Tazobactam (Rx). Available online: https://reference.medscape.com/ drug/zosyn-piperacillintazobactam-342485#10 (accessed on 7 July 2021).

43. Naka, T., & Fujimoto, M. 2018. LRG is a novel inflammatory marker clinically useful for the evaluation of disease activity in rheumatoid arthritis and inflammatory bowel disease. *Immunological medicine*, 41(2), 62–67. https://doi.org/10.1080/13497413.2018.1481582

44. Nguyen, L., Garcia, J., Gruenberg, K., & MacDougall, C. 2018. Multidrug-Resistant Pseudomonas Infections: Hard to Treat, But Hope on the Horizon?. Current infectious disease reports, 20(8), 23. https://doi.org/10.1007/s11908-018-0629-6

45. Parthiban, N., & Harish, M. 2017. A study on microbiology culture of acute appendicectomy specimen and its correlation with wound infection. *International Surgery Journal*, 4(7), 2212. https://doi.org/10.18203/2349-2902.isj2012768

46. Pedram, A., Asadian, F., & Roshan, N. 2019. Diagnostic Accuracy of Abdominal Ultrasonography in Pediatric Acute Appendicitis. Bulletin of emergency and trauma, 7(3), 278–283. https://doi.org/10.29252/beat-0703011

47. Peeters, T., Martens, S., D'Onofrio, V., Stappers, M. H. T., van der Hilst, J. C. H., Houben, B., Achten, R., Joosten, L. A. B., & Gyssens, I. C. 2020. An observational study of innate immune responses in patients with acute appendicitis. *Scientific reports*, 10(1), 17352. https://doi.org/10.1038/s41598-020-73798-3

48. Pilmis, B., Jiang, O., Mizrahi, A., Nguyen Van, J., Lourtet-Hascoët, J., Voisin, O., Le Lorc'h, E., Hubert, S., Ménage, E., Azria, P., et al. No significant difference between ceftriaxone and cefotaxime in the emergence of antibiotic resistance in the gut microbiota of hospitalized patients: A pilot study. *Int. J. Infect. Dis*. 2021, 104, 617–623. https://doi.org/10.1016/j.ijid.2021.01.025

49. Podany AB, Tsai AY, Dillon PW 2017 Acute Appendicitis in Pediatric Patients: An Updated Narrative Review. *J Clin Gastroenterol Treat* 3:042. https://doi.org/10.23937/2469-584X/1510042

50. Qin, X., Tran, B. G., Kim, M. J., Wang, L., Nguyen, D. A., Chen, Q., Song, J., Laud, P. J., Stone, G. G., & Chow, J. W. 2017. A randomised, double-blind, phase 3 study comparing the efficacy and safety of ceftazidime/avibactam plus metronidazole versus meropenem for complicated intra-abdominal infections in hospitalised adults in Asia. *International Journal of Antimicrobial Agents*, 49(5), 579–588. https://doi.org/10.1016/j.ijantimicag.2017.01.010

51. Rainer, T. H., Leung, L. Y., Chan, C., Leung, Y. K., Cheng, N. M., Lai, P., Cheung, Y. S., & Graham, C. A. 2017. Circulating human leucine-rich α-2-glycoprotein 1 mRNA and protein levels to detect acute appendicitis in patients with acute abdominal pain. *Clinical biochemistry*, 50(9), 485–490.

52. Rautava, L., Rautava, P., Sipilä, J., & Kytö, V. 2018. Occurrence and Treatment of Pediatric Appendicitis in Finland 2004-2014. *The Journal of surgical research*, 232, 33–38. https://doi.org/10.1016/j.jss.2018.06.010

53. Rawolle, T., Reismann, M., Minderjahn, M. I., Bassir, C., Hauptmann, K., Rothe, K., & Reismann, J. 2019. Sonographic differentiation of complicated from uncomplicated appendicitis. *The British journal of radiology*, 92(1099), 20190102. https://doi.org/10.1259/bjr.20190102

54. Rekomendācijas Antibakteriālo Līdzekļu Lietošanai Ķirurģiskajā Profilaksē. KS/MET-011-00. Bērnu Klīniskā Universitātes Slimīca. 2019. Available online: https://www.spkc.gov.lv/lv/media/4147/download (accessed on 29 June 2022).

55. Rickard, J., Ngarambe, C., Ndayizeye, L., Smart, B., Riviello, R., Majyambere, J. P., & Ghebre, R. G. 2018. Antibiotic use and antimicrobial resistance of surgical patients with peritonitis at a tertiary referral hospital in Rwanda. *Surgical Infections*, 19(4), 382–387. https://doi.org/10.1089/sur.2017.158

56. Rogers, M. B., Brower-Sinning, R., Firek, B., Zhong, D., & Morowitz, M. J. 2016. Acute Appendicitis in Children Is Associated With a Local Expansion of Fusobacteria. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, 63(1), 71–78. https://doi.org/10.1093/cid/ciw208

57. Salö, M., Marungruang, N., Roth, B., Sundberg, T., Stenström, P., Arnbjörnsson, E., Fåk, F., & Ohlsson, B. 2017. Evaluation of the microbiome in children's appendicitis. *International journal of colorectal disease*, 32(1), 19–28. https://doi.org/10.1007/s00384-016-2639-x

58. Sarkar, P., Yarlagadda, V., Ghosh, C., & Haldar, J. 2017. A review on cell wall synthesis inhibitors with an emphasis on glycopeptide antibiotics. *MedChemComm*, 8(3), 516–533. https://doi.org/10.1039/c6md00585c

59. Schülin S, Schlichting N, Blod C, Opitz S, Suttkus A, Stingu CS, Barry K, Lacher M, Bühligen U, Mayer S. The intra- and extraluminal appendiceal microbiome in pediatric patients: A comparative study. *Medicine (Baltimore)*. 2017 Dec;96(52):e9518. doi: 10.1097/MD.0000000000009518. PMID: 29384958; PMCID: PMC6393148.

60. Selleck, M. J., Senthil, M., & Wall, N. R. 2017. Making Meaningful Clinical Use of Biomarkers. *Biomarker insights*, 12, 1177271917715236. https://doi.org/10.1177/1177271917715236

61. Snyder, M. J., Guthrie, M., & Cagle, S. 2018. Acute Appendicitis: Efficient Diagnosis and Management. *American family physician*, 98(1), 25–33.

62. Solomkin, J., Hershberger, E., Miller, B., Popejoy, M., Friedland, I., Steenbergen, J., Yoon, M., Collins, S., Yuan, G., Barie, P. S., & Eckmann, C. 2015. Ceftolozane/Tazobactam plus metronidazole for complicated intra-abdominal infections in an eFswidira of multidrug resistance: Results from a randomized, double-blind, phase 3 trial (aspect-ciai). *Clinical Infectious Diseases*, 60(10), 1462–1471. https://doi.org/10.1093/cid/civ097

63. Terentjeva, M., Streikiša, M., Avsejenko, J., Trofimova, J., Kovaļenko, K., Elferts, D., & Bērziņš, A. 2019. Prevalence and Antimicrobial Resistance of Escherichia coli, Enterococcus spp. and the Major Foodborne Pathogens in Calves in Latvia. Foodborne pathogens and disease, 16(1), 35–41. https://doi.org/10.1089/fpd.2018.2523

64. Turel, O., Mirapoglu, S. L., Yuksel, M., Ceylan, A., & Gultepe, B. S. 2019. Perforated appendicitis in children: Antimicrobial susceptibility and antimicrobial stewardship. *Journal of Global Antimicrobial Resistance*, 16, 159–161. https://doi.org/10.1016/j.jgar.2018.09.015

65. University of California San Francisco. Pediatric Appendicitis Clinical Algorithm. Available online: https://idmp.ucsf.edu/content/pediatric-appendicitis-clinical-algorithm (accessed on 7 July 2021).

66. Wagatsuma K, Yokoyama Y, Nakase H. Role of Biomarkers in the Diagnosis and Treatment of Inflammatory Bowel Disease. *Life*. 2021; 11(12):1375. https://doi.org/10.3390/life11121375

67. Wagenlehner, F. M., Umeh, O., Steenbergen, J., Yuan, G., & Darouiche, R. O. 2015. Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary-tract infections, including pyelonephritis: A randomised, double-blind, phase 3 trial (ASPECT-cUTI). *The Lancet*, 385(9981), 1949–1956. https://doi.org/10.1016/s0140-6736(14)62220-0

68. Wu Y, Wang M, Zhu Y, Lin S. Serum interleukin-6 in the diagnosis of bacterial infection in cirrhotic patients: A meta-analysis. *Medicine (Baltimore)*. 2016 Oct;95(41): e5127. doi: 10.1097/MD.0000000000005127. PMID: 27741137; PMCID: PMC5072964.

69. Yap, T. L., Fan, J. D., Chen, Y., Ho, M. F., Choo, C. S., Allen, J., Low, Y., Jacobsen, A. S., & Nah, S. A. 2019. A novel noninvasive appendicitis score with a urine biomarker. *Journal of pediatric surgery*, 54(1), 91–96. https://doi.org/10.1016/j.jpedsurg.2018.10.025

Acknowledgments

I would like to express my deepest gratitude to my supervisors Professor Juta Kroiča and Professor Arnis Enģelis, who generously shared their knowledge and expertise, support, patience, advice, and encouragement throughout process of developing this Doctoral Thesis.

I am extremely grateful to my scientific advisors Professor Amulya Saxena and Assistant Professor Aigars Reinis for all the guidance, support, and outstanding feedback whenever it was needed during this journey.

I would like to express my sincere gratitude to Professor Aigars Pētersons for the generous support in the process of creating scientific publications and Ph.D. thesis, sharing his experience, support, and knowledge.

Special thanks to Lāsma Asare and Renārs Broks for data statistical processing.

Finally, I would like to thank the patients, their parents or guardians who agreed to participate in this study and every single person who participated and helped in this whole process including the Latvian Council of Science and Rīga Stradiņš University for their sincere contribution in funding this project. I am very grateful for all the help and support from so many people who have participated in the whole process of developing this Doctoral Thesis. I could not have undertaken this journey without the help of my colleagues, students, friends, and family.

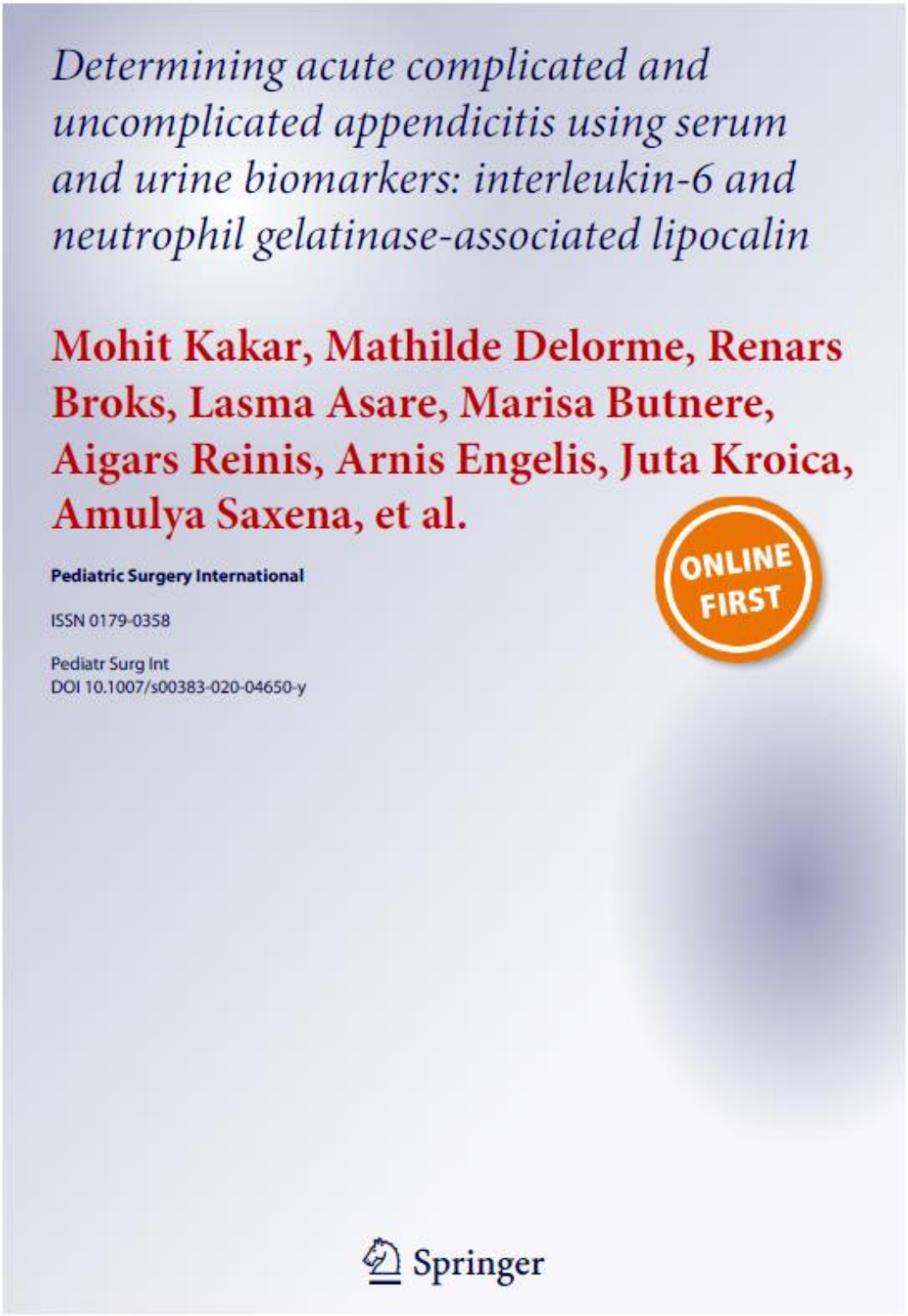
To our knowledge, this is the first time that these biomarkers serum and urine IL-6, NGAL and LRG1 have been compared together in a complex study for the diagnosis of paediatric acute appendicitis and differentiation between AcA and AuA.

According to the results of this novelty study, S-NGAL, S-LRG1, U-LRG1 and S-IL-6 may be effective biomarkers in the differential diagnosis of acute abdominal pain in children presenting to the emergency department, depending on the onset of symptoms.

2 hypotheses were made and both were confirmed in this study, because 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, as seen in the results obtained. And the microbiota in the appendix can promote the management of acute complicated and uncomplicated appendicitis in paediatric patients, as data were obtained on different microorganisms for each group of patients, as well as on their sensitivity and resistance to different antibacterial agents.

Annexes

First Publication



Your article is protected by copyright and all rights are held exclusively by Springer-Verlag GmbH Germany, part of Springer Nature. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Springer

Author's personal copy

Pediatric Surgery International
https://doi.org/10.1007/s00383-020-04650-y

ORIGINAL ARTICLE

Determining acute complicated and uncomplicated appendicitis using serum and urine biomarkers: interleukin-6 and neutrophil gelatinase-associated lipocalin

Mohit Kakar[2,4] · Mathilde Delorme[5] · Renars Broks[1] · Lasma Asare[6] · Marisa Butnere[2,4] · Aigars Reinis[1] · Arnis Engelis[2,4] · Juta Kroica[1] · Amulya Saxena[3] · Aigars Petersons[2,4]

Accepted: 5 March 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Purpose The study aim is to determine whether serum and urine interleukin-6 (IL-6) and neutrophil gelatinase-associated lipocalin (NGAL) can be included in the early diagnostic algorithm for pediatric appendicitis.

Methods Prospective single-center cohort study included 92 children divided into control, acute complicated appendicitis (AcA) and acute uncomplicated appendicitis (AnA) groups. Serum and urine samples were assayed for IL-6 and NGAL preoperatively, and on the second and fifth postoperative days. Intraoperative and bacteriological findings divided the appendicitis patients.

Results Average serum biomarker levels were higher in appendicitis patients versus the control, and the following values were produced via receiver operating characteristic (ROC) analysis. NGAL and IL-6 cutoff values were 113.95 ng/ml and 24.64 pg/ml, respectively, NGAL had 68.3% sensitivity and 65.5% specificity, while IL-6 had 72.6% and 86.2%. Comparing AcA and AnA, IL-6 was the only biomarker of significance yielding 77.4% sensitivity and 58.1% specificity with a 26.43 pg/ml cutoff value. Urine biomarkers were non-specific in differentiation appendicitis severity and ultimately, between infectious and non-infectious disease.

Conclusion Although NGAL provided measurable useful diagnostic information in evaluating children for appendicitis, its values were not sufficient for appendicitis severity. Serum IL-6 remains a strong biomarker for suspected acute appendicitis and has promising results predicting its severity.

Keywords Pediatric appendicitis · Appendectomy · Biomarkers · NGAL · IL-6

Introduction

Acute appendicitis is one of the most common pediatric abdominal pathologies that require surgery, and despite advances in diagnostic tests, it still predominantly relies on typical clinical findings and patient medical history [1, 2].

It is particularly difficult to collect the patient history of young children, who may be less cooperative and a less accurate source of information [1–4]. Various laboratory values such as leukocytosis, increased C-reactive protein, and imaging such as abdominal ultrasound and computer tomography have proven useful in decreasing the rate of unnecessary diagnostic laparoscopies [5]. Laparoscopy is considered a diagnostic method for abdominal pain of unclear etiology and suspected appendicitis. If an inflamed appendix is left untreated, it could progress to peritonitis, abscess, sepsis and death [6–8]. Nowadays, acceptable

✉ Mohit Kakar
mohitez@gmail.com

[1] Department of Biology and Microbiology, Riga Stradins University, Dzirciema iela, 16, Riga 1007, Latvia

[2] Department of Pediatric Surgery, Children's Clinical University Hospital, Vienibas gatve, 45, Riga 1004, Latvia

[3] Department of Pediatric Surgery, Imperial College London, Chelsea Children's Hospital, Chelsea and Westminster NHS Foundation Trust, London, UK

[4] Department of Pediatric Surgery, Riga Stradins University, Dzirciema iela, 16, Riga, Latvia

[5] Faculty of Medicine, Riga Stradins University, Dzirciema iela, 16, Riga, Latvia

[6] Department of Statistics, Riga Stradins University, Dzirciema iela, 16, Riga, Latvia

Published online: 26 March 2020

Author's personal copy

negative appendectomy rates vary from 4 to 45% with a high incidence among women of reproductive age, and 3.6–21.3% in children depending on age and gender [6, 9].

Over the past decade, attempts have been made to limit the use of computed tomography due to long-term cancer risk and to decrease the rate of unnecessary surgeries and overall complications [4]. The introduction of novel biomarkers as part of the diagnostic criteria offers a non-invasive method that can yield similar information and diagnostic accuracy [10]. Interleukin-6 (IL-6) is a highly multifunctional inflammatory marker, the formation of which is highly dependent on tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). Its functions are vastly diverse, including initiation of acute-phase protein synthesis in the liver, activation of hemopoiesis, activation of B-cells, and participation in the formation of Th17 [11–13]. IL-6 is found in large amounts in patients with sepsis. Recent literature has revealed that this marker is an ideal biomarker for bacterial infections and could serve as an early rapid diagnostic tool in clinically suspected appendicitis [2, 12]. Neutrophils are primary respondents to inflammation and, if their count in the bloodstream is increased, then this could also mean an increase in neutrophil gelatinase-associated lipocalin (NGAL). Renal system, respiratory and gastrointestinal tract tissues express low levels of NGAL. Serum NGAL levels appear to increase in correlation with epithelial damage under stress, for instance processes such as inflammation, infection and ischemia [14–16]. Thus, theoretically both IL-6 and NGAL serum levels would be higher in acute complicated appendicitis (AcA) rather than in acute uncomplicated appendicitis (AnA), in which the organ tissues are subject to less stress.

Focus on immunological pathways is expanding, and consequently the magnitude of proposed biomarkers, although none have currently achieved widespread use [16]. The search for the optimal biomarker may be futile, but in combination with medical history and clinical findings, it is possible to improve the quality of diagnostic approaches; therefore, decrease complications, and reduce the overall costs incurred by the hospital as the result of a reduction in unnecessary imaging and surgeries. Our primary objective was to demonstrate the potential of the inflammatory protein mediator NGAL and IL-6 in a prospective cohort study of children with suspected appendicitis—AcA and AnA.

Methods

This study included children between the ages of 7 and 17 admitted to the Children's Clinical University Hospital in Riga due to acute abdominal pain with signs and symptoms suggesting the possibility of appendicitis. All patients were examined to confirm or exclude the diagnosis. Preoperative screening involved physical examination, complete blood count, abdominal ultrasound (US) and detection of serum values of C-reactive protein (CRP) and interleukin-6 (IL-6). Depending on the intraoperative and bacteriological findings, two groups were established—acute complicated appendicitis (AcA) and acute uncomplicated appendicitis (AnA). In addition, a third group was created, consisting of control patients without any suspected inflammatory processes in the renal system or respiratory or gastrointestinal tracts. Therefore, patients in the control group mainly consisted of those admitted to the orthopedic emergency department. The pediatric surgery team supervising these patients received a written consent form from the caregiver and assent from the patient if they were 13 years of age or older. With this consent form, the patient and caregiver were informed about the research objective and methodology of the biological material. A total of 92 patients were eligible for and included in our research—32 AcA, 31 AnA and 29 Control.

Sample collection

On the day of hospital admission, all patient blood and urine samples were taken. The necessary amount of blood per patient for IL-6 and NGAL was 300 µl and 100 µl, respectively. After centrifugation serum was collected and stored at − 80 °C. Midstream, clean-catch urine specimens (IL-6 200 µl; NGAL 100 µl) were collected and centrifuged at 2000 rpm for 10 min at 4 °C as to remove cell debris from urine. The supernatant was stored at − 80 °C before the moment of analysis. Appendicitis patient samples were subsequently collected on the second and fifth postoperative day.

Measurement of NGAL and IL-6 levels

NGAL and IL-6 concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine® ELISA, human lipocalin-2/NGAL and Quantikine® ELISA, human IL-6; R&D Systems, Minneapolis, MN, USA). All procedures performed on these concentrations were applied per the manual of these kits. To secure experiment quality, IL-6 (Immunoassay Control Group 1 QC01-1, R&D System Inc., Minneapolis, MN, USA) and NGAL (Immunoassay Control Set QC115, R&D System Inc., Minneapolis, MN, USA) controls were used. All samples were measured in triplets and each individual result was obtained in approximately 2–3 h.

Statistical analysis

For statistical analysis, Microsoft Excel 2016 and IBM SPSS Statistics 22 were utilized. Results were expressed as median

Author's personal copy

values and interquartile ranges (IQR). The comparisons between groups were calculated using both the Mann–Whitney *U* test (2 groups) and Kruskal–Wallis test (3 groups) for non-parametric distribution, while Fisher exact test was applied on normally distributed variables to determine correlations between them. The receiver operating characteristic (ROC) curve determined the clinical importance of the biomarkers and their diagnostic value regarding acute appendicitis. A p value of <0.05 was considered statistically significant.

Results

The study included 92 children—42 boys and 50 girls. Patients' ages ranged from 7 to 17 years, with an average of 12.3 ± 3.1 years. From these 92 patients, 63 (68.5%) were diagnosed with appendicitis and 29 (31.5%) had no infectious or inflammation-mediated pathologies. Suspicion of appendicitis required an urgent diagnostic laparoscopy in 53 (84.1%) patients and laparotomy in 10 (15.9%) of the cases.

Intraoperative swabs of free abdominal liquid were collected, establishing the presence of bacterial growth in the abdominal cavity. Hence, acute complicated and acute uncomplicated appendicitis were differentiated based on the growth of bacteria. Positive-growth established the AcA group of 32 (50.7%) and no growth established AnA group of 31 (49.2%).

More than half of patients (61.3%) that had a drainage tube inserted were diagnosed with AcA ($p=0.004$). A simple comparison suggests that AnA had a slightly shorter median postoperative hospital stay, 5 versus 6 days. The distribution of the study population is shown in Table 1.

Baseline preoperative values of IL-6 and NGAL are presented in Table 2, along with the values of the second and the fifth postoperative days. The lowest baseline level (day 0) of all observed parameters was found in the control group without the infectious disease, whilst the highest was observed in AcA. The median serum NGAL 0 levels for AcA, AnA and control group were 169.90 ng/ml, 133.70 ng/mL, and 90.14 ng/ml, respectively. The distribution of S-NGAL (serum-NGAL) 0 was statistically higher (T-stat = 11.023, $p=0.004$) in AcA compared to the control group ($p=0.003$). NGAL values of AnA were higher than those of the control group. Thus, the NGAL values of AcA were the highest.

NGAL levels on the second postoperative day decreased to 107.18 ng/mL in AcA and 75.12 ng/ml in AnA ($p<0.001$). Although the values were significantly different on the second postoperative day ($p<0.001$), there was no significant difference between the NGAL values in AcA and AnA preoperatively ($p=0.612$) (Table 2, Fig. 1).

The baseline distribution of serum IL-6 was statistically significant across AcA, AnA and control group. The average distribution of serum IL-6 differed significantly between the diagnostic groups, T-stat = 28.967, $p<0.001$. The difference was seen between the control group vs. AnA ($p=0.009$), between control group vs. AcA ($p<0.001$), as well as the difference between AnA vs AcA ($p=0.045$).

A drastic decrease in the levels of biomarkers can be observed from day 0 until day 5, as the inflammation settles postoperatively; it is presented in Table 2 and demonstrated

Table 1 Characteristics and distribution of study population

	AcA $n=32$	AnA $n=31$	Ctrl[a] $n=29$	Total $n=92$	p value
Gender, n (%)					
Boy	15 (16.3)	17 (18.5)	18 (19.5)	50 (54.3)	0.492
Girl	17 (18.6)	14 (15.2)	11 (11.9)	42 (45.7)	
Age ± SD	12.3 ± 3.1	12.3 ± 3.1	12.3 ± 3.1		
Type of surgery, n (%)					
Laparotomy	7 (21.2)	3 (10.0)		10 (15.9)	0.201
Laparoscopy	26 (78.8)	7 (90.0)		53 (84.1)	
Drainage tube, n (%)					
Yes	19 (61.3)	7 (24.1)		26 (43.3)	0.004
No	12 (38.7)	22 (75.9)		44 (56.7)	
Length of hospital stay, days (IQR)	6 (5–9)	5 (4–6)			0.221

Median values are presented with IQR (25%, 75%)

AcA acute complicated appendicitis, *AnA* acute uncomplicated appendicitis, *Ctrl* control, *NGAL* neutrophil gelatinase-associated lipocalin, *LRG* leucine-rich alpha glycoprotein-1, *IL-6* interleukin-6

[a]The Control group did not undergo abdominal surgery

Author's personal copy

negative appendectomy rates vary from 4 to 45% with a high incidence among women of reproductive age, and 3.6–21.3% in children depending on age and gender [6, 9].

Over the past decade, attempts have been made to limit the use of computed tomography due to long-term cancer risk and to decrease the rate of unnecessary surgeries and overall complications [4]. The introduction of novel biomarkers as part of the diagnostic criteria offers a non-invasive method that can yield similar information and diagnostic accuracy [10]. Interleukin-6 (IL-6) is a highly multifunctional inflammatory marker, the formation of which is highly dependent on tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β). Its functions are vastly diverse, including initiation of acute-phase protein synthesis in the liver, activation of hemopoiesis, activation of B-cells, and participation in the formation of Th17 [11–13]. IL-6 is found in large amounts in patients with sepsis. Recent literature has revealed that this marker is an ideal biomarker for bacterial infections and could serve as an early rapid diagnostic tool in clinically suspected appendicitis [2, 12]. Neutrophils are primary respondents to inflammation and, if their count in the bloodstream is increased, then this could also mean an increase in neutrophil gelatinase-associated lipocalin (NGAL). Renal system, respiratory and gastrointestinal tract tissues express low levels of NGAL. Serum NGAL levels appear to increase in correlation with epithelial damage under stress, for instance processes such as inflammation, infection and ischemia [14–16]. Thus, theoretically both IL-6 and NGAL serum levels would be higher in acute complicated appendicitis (AcA) rather than in acute uncomplicated appendicitis (AnA), in which the organ tissues are subject to less stress.

Focus on immunological pathways is expanding, and consequently the magnitude of proposed biomarkers, although none have currently achieved widespread use [16]. The search for the optimal biomarker may be futile, but in combination with medical history and clinical findings, it is possible to improve the quality of diagnostic approaches; therefore, decrease complications, and reduce the overall costs incurred by the hospital as the result of a reduction in unnecessary imaging and surgeries. Our primary objective was to demonstrate the potential of the inflammatory protein mediator NGAL and IL-6 in a prospective cohort study of children with suspected appendicitis—AcA and AnA.

Methods

This study included children between the ages of 7 and 17 admitted to the Children's Clinical University Hospital in Riga due to acute abdominal pain with signs and symptoms suggesting the possibility of appendicitis. All patients were examined to confirm or exclude the diagnosis. Preoperative screening involved physical examination, complete blood count, abdominal ultrasound (US) and detection of serum values of C-reactive protein (CRP) and interleukin-6 (IL-6). Depending on the intraoperative and bacteriological findings, two groups were established—acute complicated appendicitis (AcA) and acute uncomplicated appendicitis (AnA). In addition, a third group was created, consisting of control patients without any suspected inflammatory processes in the renal system or respiratory or gastrointestinal tracts. Therefore, patients in the control group mainly consisted of those admitted to the orthopedic emergency department. The pediatric surgery team supervising these patients received a written consent form from the caregiver and assent from the patient if they were 13 years of age or older. With this consent form, the patient and caregiver were informed about the research objective and methodology of the biological material. A total of 92 patients were eligible for and included in our research—32 AcA, 31 AnA and 29 Control.

Sample collection

On the day of hospital admission, all patient blood and urine samples were taken. The necessary amount of blood per patient for IL-6 and NGAL was 300 µl and 100 µl, respectively. After centrifugation serum was collected and stored at − 80 °C. Midstream, clean-catch urine specimens (IL-6 200 µl; NGAL 100 µl) were collected and centrifuged at 2000 rpm for 10 min at 4 °C as to remove cell debris from urine. The supernatant was stored at − 80 °C before the moment of analysis. Appendicitis patient samples were subsequently collected on the second and fifth postoperative day.

Measurement of NGAL and IL-6 levels

NGAL and IL-6 concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Quantikine® ELISA, human lipocalin-2/NGAL and Quantikine® ELISA, human IL-6; R&D Systems, Minneapolis, MN, USA). All procedures performed on these concentrations were applied per the manual of these kits. To secure experiment quality, IL-6 (Immunoassay Control Group 1 QC01-1, R&D System Inc., Minneapolis, MN, USA) and NGAL (Immunoassay Control Set QC115, R&D System Inc., Minneapolis, MN, USA) controls were used. All samples were measured in triplets and each individual result was obtained in approximately 2–3 h.

Statistical analysis

For statistical analysis, Microsoft Excel 2016 and IBM SPSS Statistics 22 were utilized. Results were expressed as median

Author's personal copy

Table 2 Preoperative and postoperative biomarker levels per appendix status

Biomarker	AcA, ng or pg/ml (IQR)	AnA, ng or pg/ml (IQR)	Ctrl, ng or pg/ml (IQR)*	*p* value
Day 0				
Serum NGAL	169.90 (104.58–254.25)	133.70 (89.50–188.20)	90.14 (70.80–139.15)	0.004
Urine NGAL	3.39 (1.51–8.42)	4.19 (1.44–8.90)	3.14 (1.47–6.59)	0.846
Serum IL-6	48.39 (26.62–132.98)	22.57 (11.67–48.47)	10.30 (4.97–18.53)	<0.001
Urine IL-6	11.22 (4.21–31.98)	20.570 (1.82–65.37)	18.93 (2.77–100.49)	0.568
Day 2				
Serum NGAL	107.18 (81.50–169.30)	75.12 (60.53–88.43)		<0.001
Urine NGAL	2.85(0.92–7.31)	3.88 (1.67–16.00)		0.222
Serum IL-6	11.33 (6.61–33.04)	7.11 (2.37–29.90)		0.102
Urine IL-6	15.14 (3.36–31.58)	5.86 (2.07–18.80)		0.159
Day 5				
Serum NGAL	85.33 (65.52–103.81)	69.34 (58.26–91.10)		0.174
Urine NGAL	2.80 (1.22–5.87)	5.68 (1.27–30.18)		0.088
Serum IL-6	4.41 (1.98–15.67)	5.51 (2.54–21.88)		0.322
Urine IL-6	8.26 (2.16–102.09)	3.79 (1.73–11.34)		0.109

Biomarker levels are expressed as medians, IQR (25%, 75%)

IL-6 is measured in pg/ml and NGAL in ng/ml

AcA acute complicated appendicitis, *AnA* acute uncomplicated appendicitis, *Ctrl* control, *NGAL* neutrophil gelatinase-associated lipocalin, *IL-6* interleukin-6

*The group did not undergo abdominal surgery; thus, only biomarkers day 0 are included in the study.

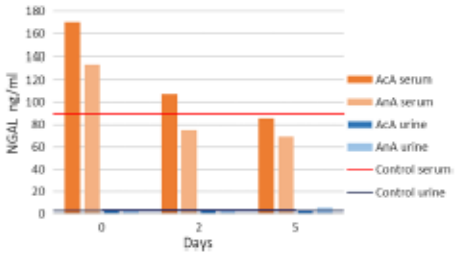


Fig. 1 Serum NGAL levels on operative day (0), 2nd postoperative day (2) and 5th postoperative day (5) in patients with AcA, AnA compared to the control group. Difference in serum NGAL values were significantly higher ($p<0.004$) between AcA and Control on the operation day (0)

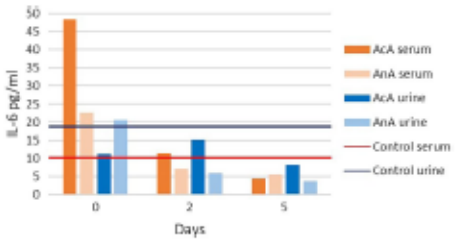


Fig. 2 Serum IL-6 levels on operative day (0), 2nd post-operative day (2) and 5th post-operative day (5) in patients with AcA, AnA and Ctrl. Serum NGAL values were significantly higher ($p<0.001$) between AcA and AnA; between AcA and Ctrl on the operative day (0)

in Fig. 2. The median S-IL-6 0 levels were 48.39 pg/ml (AcA), 22.57 pg/ml (AnA) and 10.30 pg/ml (Control).

If AcA and AnA were categorized as the same type of "appendicitis" and compared to the control group, a significant difference between the baseline NGAL and IL-6 individually ($p=0.002$; $p<0.001$) (Table 3) could be seen.

The urine samples of both IL-6 and NGAL were non-informative and, thus, not specific to differentiate between AcA and AnA or between infectious disease and non-infectious disease (Table 2).

The NGAL cutoff value for patients with appendicitis was 113.95 ng/ml and IL-6 cutoff was 21.64 pg/ml ($p=0.002$; $p<0.001$). The ROC curves demonstrated AUC 0.70 (95% CI 0.59–0.81) and AUC 0.73 (95% CI 0.73–0.90), respectively (Figs. 3a, 4a). NGAL for appendicitis had a sensitivity of 68.3% and specificity of 65.5%, while IL-6 showed a higher sensitivity and specificity of 72.6% and 86.2%. The efficiency of NGAL as a predictive biomarker AcA versus AnA was found to be insignificant ($p=0.461$) AUC 0.60 (95% CI 0.46–0.74). Contrarily, IL-6 was a good predictive biomarker for determining whether the patient had AcA ($p<0.001$) AUC 0.70 (95% CI 0.56–0.83) with a cutoff value at 26.43 pg/ml and a sensitivity of 77.4% and specificity of 58.1%.

Author's personal copy

Table 3 Evaluating pre-operative biomarker levels of appendicitis vs control group

Biomarker	Appendicitis ng or pg/ml (IQR)	Ctrl, ng or pg/ml (IQR)	*p*
Day 0			
Serum NGAL	144.80 (104.80–244.30)	90.14 (70.80–139.15)	0.002
Urine NGAL	3.69 (1.71–8.86)	3.23 (1.58–6.84)	0.721
Serum IL-6	32.91 (21.45–95.36)	10.93 (5.89–18.53)	< 0.001
Urine IL-6	14.64 (3.02–36.17)	16.57 (2.37–141.50)	0.298

Biomarker levels are expressed as medians, IQR (25%, 75%)

p values were derived using Mann–Whitney *U* Test

IL-6 is measured in pg/ml and NGAL in ng/ml

Appendicitis AcA and AnA, *Ctrl* control, *NGAL* neutrophil gelatinase-associated lipocal, *IL-6* interleukin-6

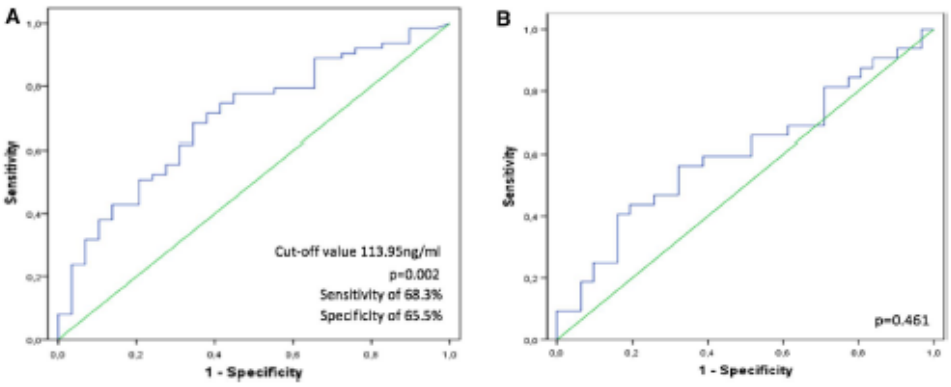


Fig. 3 The ROC curve for NGAL in appendicitis vs. non-appendicitis patients demonstrates AUC 0.70 (95% CI 0.59–0.81) at a cutoff value of 113.95 ng/ml (**a**). ROC curve for AnA versus AcA patients demonstrates AUC 60.2 (95% CI 0.46–0.74) (**b**)

Discussion

Appendicitis continues to have a major diagnostic challenge, which heavily relies on clinical assessment of patients and scoring systems to guide diagnosis. Modern diagnostic methods are utilized with the aim of confirming the diagnosis of appendicitis and to predict its course and severity [17]. None of the inflammatory markers such as white blood cell count, C-reactive protein and procalcitonin have been established as a singular marker ensuring high specificity or sensitivity in the diagnosis of appendicitis [17, 18].

In this study, two novel biomarkers were assessed in pediatric patients with acute appendicitis. Their marked expression is believed to represent the inflammatory process occurring in patients with appendicitis [14–16, 19]. Neutrophil gelatinase-associated lipocalin (NGAL) also known as Lipocalin-2 (LCN2) and oncogene 24p3 is a 20 kDa protein that is encoded by the LCN2 gene in humans [19]. NGAL is involved in the innate immunity by sequestrating iron that in turn limits bacterial growth. It is expressed in neutrophils and in low levels in the renal system, and gastrointestinal and respiratory tracts [19]. When one or more of these organ tissues experience epithelial damage, the serum level of NGAL temporarily increases. NGAL is secreted in high levels in the blood within 2 h of injury [19]. Since NGAL is protease resistant and small, the protein is easily excreted and detected in the urine. An NGAL level in patients with acute kidney injury (AKI) has been associated with the severity of their prognosis and is widely used as a biomarker for kidney injury.

We found that NGAL levels are increased in patients with acute appendicitis pre-operatively and low in patients without any abdominal inflammation, which supports results found by Bakal et al. [14]. NGAL was useful to clinically distinguish patients who had appendicitis from patients who did not have infectious or inflammation-mediated pathologies.

Author's personal copy

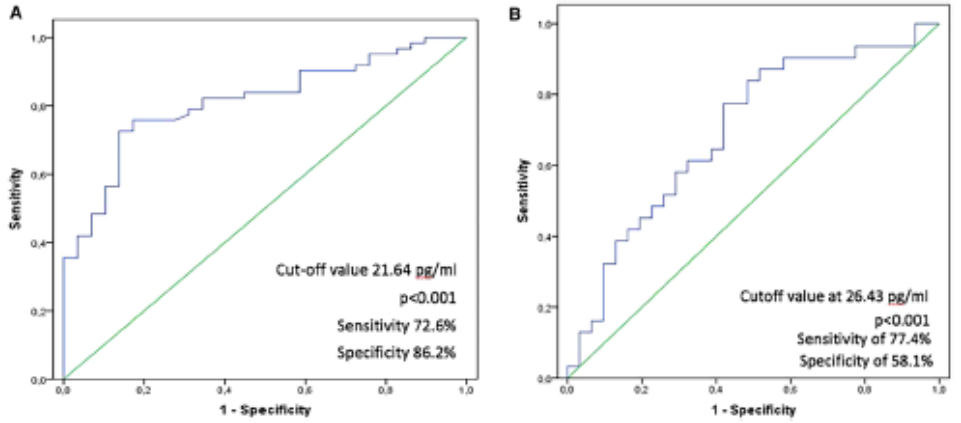


Fig. 4 The ROC curve for IL-6 appendicitis vs. non-appendicitis patients demonstrate AUC 0.73 (95% CI 0.73–0.90) at a cutoff value of 21.64 pg/ml (**a**). ROC curve for AnA vs. AcA patients demonstrates AUC 0.70 (95% CI 0.56–0.83) at a cutoff value of 26.43 pg/ml (**b**)

In contrast to other studies, our study also aimed to measure the severity of appendicitis by subdividing cases into AcA and AnA. However, serum N-GAL (S-NGAL) was not a good biomarker to detect the severity of AcA. This may be due to biomarkers having a limited time frame of utility, which could influence diagnostic accuracy. Ataie et al. suggested that the diagnosis of AKI investigating NGAL levels is more accurate within a time frame of 12 h following the onset of injury [19]. Another study revealed an early increase of IL-6 immediately after tissue trauma (within 6 h) and an increase in C-reactive protein levels at a later stage of epithelial damage (within 12 h) [20]. In conclusion, NGAL could be used as a predictive biomarker in the early stages or for establishing diagnosis, however, its level decreases within a few days. Therefore, the time frame for using NGAL necessitates further research to evaluate when the biomarker has greater diagnostic value.

Unlike S-NGAL, the difference in serum IL-6 was significant among all three groups. Serum IL-6 in AcA was five times higher than the control group and three times higher than AnA. This difference in IL-6 levels between AcA and AnA can determine the severity of appendicitis. Regarding the clinical course of appendicitis, we found that patients had a significantly lower level of serum NGAL and IL-6 on their fifth postoperative day than before surgery. Postoperatively, biomarkers declined to the level of those in the control group.

The fact that certain individuals within the control group had slightly elevated values, could potentially suggest a temporary epithelial inflammation in the renal system, gastrointestinal or respiratory tracts [17–19].

Non-invasive diagnostic markers are exceptionally attractive in pediatrics; however, they are rarely applied due to patient compliance issues and lack of evidence that urine biomarkers can be of diagnostic value. Although we assayed NGAL and IL-6 in urine, no significant difference was observed between patients with appendicitis and without appendicitis. Ataei et al. showed promising results of elevated urine-NGAL (U-NGAL) in case of pediatric AKI within 12 h of onset of injury [19]. The predictive and reliable value of U-NGAL in AKI is based on the increased production of NGAL in renal tubules and subsequent release into urine. According to our study, U-NGAL levels in urine were not significant in patients with appendicitis and, therefore, cannot be used in the assessment and diagnosis of appendicitis [19]. Results received by Oikonomou et al. from their investigation of U-NGAL and S-NGAL (NGAL concentration in serum) in inflammatory bowel disease, confirm this statement [21]. In the absence of pathology, NGAL is detected in the systemic circulation and kidneys. It is filtered through the glomerulus and luminal NGAL is then reabsorbed from the proximal tubule by the megalin-dependent pathway [21–23]. In AKI, NGAL is upregulated at the distal part of the nephron impairing the reabsorption pattern in the tubules, and leading to elevated U-NGAL and S-NGAL [19, 22, 23]. Renal function is not impaired in patients with appendicitis; therefore, NGAL is reabsorbed within the standard range. Since our study reported negative results regarding urine biomarkers, we believe that our results have significant value towards the clinical prediction of appendicitis and that these and other non-invasive biomarkers could be of importance in the near future's research.

Author's personal copy

Our study has some limitations that deserve mentioning. A single-centered institution with limited number of resources during odd hours and need for consent of patients along with their parents constrained the number of participants. Future research projects need to include a larger cohort population. The investigation of NGAL as a biomarker in pediatric appendicitis requires more research. In prospective studies, it should be stressed that biomarkers could have different levels within the time frame of pathology and, therefore, variable results between patients presenting to the emergency department at different times after onset. Future studies must consider other inflammatory biomarkers to increase diagnostic efficacy of severity of appendicitis.

Conclusion

Serum neutrophil gelatinase-associated lipocalin can be an effective biomarker in the differential diagnosis of acute abdominal pain in children presenting to the emergency department. It can be helpful to distinguish between suspected appendicitis and non-infectious abdominal diseases. The use of biomarkers will not alter the clinical evaluation; however, it can be a useful addition in the diagnosis of complicated and uncomplicated appendicitis. Its application could serve to improve cost effectiveness of diagnostic approaches in children who are suspected to have acute appendicitis, however, not to differentiate the severity in an acute setting. Further research would be of value to evaluate NGAL's dynamics to gain an understanding of when and how to use this biomarker. Interleukin-6 can be an effective predictive biomarker for differentiating among patients with acute complicated appendicitis and those with acute uncomplicated appendicitis.

Acknowledgements The authors would like to acknowledge every single person, the Ethics Committee, the Latvian Council of Science, and Riga Stradins University, for their sincere contribution in funding this project.

Funding This study was funded by Latvian Academy of Science and Riga Stradins University.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Riga Stradins University; reference number: 21/27.04.2017. and Children's Clinical University Hospital; reference number: SP-37/2018.) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from a parent of each individual participant included in the study.

References

1. Kingsnorth A, Reilly DO (2006) Clinical review acute pancreatitis. BMJ 332:1072–1076. https://doi.org/10.1136/bmj.332.7549.1072
2. Kharbanda AB, Rai AJ, Cosme Y, Liu K, Dayan PS (2012) Novel serum and urine markers for pediatric appendicitis. Acad Emerg Med 19(1):56–62. https://doi.org/10.1111/j.1553-2712.2011.01251.x
3. Almaramhy HH (2017) Acute appendicitis in young children less than 5 years: review article. Ital J Pediatr 43:1–9. https://doi.org/10.1186/s13052-017-0335-2
4. Wray CJ, Kao LS, Millas SG, Tsao K, KoTC (2013) Acute appendicitis: controversies in diagnosis and management. Curr Probl Surg 50(2):54–86. https://doi.org/10.1067/j.cpsurg.2012.10.001
5. Coursey CA, Nelson RC, Patel MB, Cochran C, Dodd LG, DeLong DM, Vaslef S (2010) Making the diagnosis of acute appendicitis: do more preoperative CT scans mean fewer negative appendectomies? A 10-year study. Radiology 254(2):460–468. https://doi.org/10.1148/radiol.09082298
6. Joshi MK, Joshi R, Alam SE, Agarwal S, Kumar S (2015) Negative appendectomy: an audit of resident-performed surgery. How can its incidence be minimized? Indian J Surg 77:913–917. https://doi.org/10.1007/s12262-014-1063-0
7. D'Souza N, Nugent K (2014) Appendicitis. BMJ Clin Evid 2014:0408 **(PMID: 25486014; PMCID: PMC4259213)**
8. Blomqvist PG, Andersson REB, Granath F, Lambe MP, Ekbom AR (2001) Mortality after appendectomy in Sweden, 1987–1996. Ann Surg 233(4):455–460. https://doi.org/10.1097/00000658-200104000-00001
9. Bachur RG, Hennelly K, Callahan MJ, Chen C, Monuteaux MC (2012) Diagnostic imaging and negative appendectomy rates in children: effects of age and gender. Pediatrics 129(5):877–884. https://doi.org/10.1542/peds.2011-3375
10. Kentsis A, Lin YY, Kurek K, Calicchio M, Wang YY, Monigatti F, Bachur R (2010) Discovery and validation of urine markers of acute pediatric appendicitis using high-accuracy mass spectrometry. Ann Emerg Med. https://doi.org/10.1016/j.annemergmed.2009.04.020
11. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, Heinrich PC (1989) Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett 242(2):237–239. https://doi.org/10.1016/0014-5793(89)80476-4
12. Wu Y, Wang M, Zhu Y, Lin S (2016) Serum interleukin-6 in the diagnosis of bacterial infection in cirrhotic patients: a meta-analysis. Medicine (United States) 95(41):2–7. https://doi.org/10.1097/MD.0000000000005127
13. Rincon M (2012) Special issue on interleukin-6 (IL-6). Int J Biol Sci 8(9):1225–1226. https://doi.org/10.7150/ijbs.8.1225
14. Bakal U, Saraç M, Ciftci H, Tartar T, Kocdemir E, Aydin S, Kazez A (2016) Neutrophil gelatinase-associated lipocal in protein levels as an acute appendicitis biomarker in children. SpringerPlus 5(1):1–5. https://doi.org/10.1186/s40064-016-1853-x
15. Bianca G, Raluca F, Veres M, Orlandea M, Badea J, Hlavathy K, Cioc A (2015) Plasma neutrophil gelatinase associated lipocalin (NGAL)—early biomarker for acute kidney injury in

Author's personal copy

critically ill patients. J Crit Care Med 1(4):154–161. https://doi.org/10.1515/jccm-2015-0023
16. Selleck MJ, Senthil M, Wall NR (2017) Making meaningful clinical use of biomarkers. Biomark Insights 12:1–7. https://doi.org/10.1177/1177271917715236
17. Podany AB (2017) Acute appendicitis in pediatric patients: an updated narrative review. J Clin Gastroenterol Treat 3(1):1–9. https://doi.org/10.23937/2469-584x/1510042
18. Alvarado A (2018) Inflammatory markers in acute appendicitis: are we still looking for the Philosopher's stone? J Surg 2018:1104. https://doi.org/10.29011/2575-9760.001104
19. Kari JA, Shalaby MA, Sofyani K et al (2018) Urinary neutrophil gelatinase-associated lipocalin (NGAL) and serum cystatin C measurements for early diagnosis of acute kidney injury in children admitted to PICU. World J Pediatr 14(2):134–142. https://doi.org/10.1007/s12519-017-0110-x
20. Gebhard F, Pfetsch H, Steinbach G, Strecker W, Kinzl L, Brückner UB (2000) Is interleukin 6 an early marker of injury severity following major trauma in humans? Arch Surg 135(3):291–295. https://doi.org/10.1001/archsurg.135.3.291
21. Oikonomou KA, Kapsoritakis AN, Theodoridou C, Karangelis D, Germenis A, Stefanidis I, Potamianos SP (2012) Neutrophil gelatinase-associated lipocalin (NGAL) in inflammatory bowel disease: association with pathophysiology of inflammation, established markers, and disease activity. J Gastroenterol 47(5):519–530. https://doi.org/10.1007/s00535-011-0516-5
22. Schmidt-Ott KM, Mori K, Jau YL, Kalandadze A, Cohen DJ, Devarajan P, Barasch J (2007) Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 18(2):407–413. https://doi.org/10.1681/ASN.2006080882
23. Singer E, Markó L, Paragas N, Barasch J, Dragun D, Müller DN, Schmidt-Ott KM (2013) Neutrophil gelatinase-associated lipocalin: pathophysiology and clinical applications. Acta Physiol 207(4):663–672. https://doi.org/10.1111/apha.12054
24. Andersson R, Hugander A, Thulin A, Nystrom PO, Olaison G (1994) Indications for operation in suspected appendicitis and incidence of perforation. BMJ 308(6921):107. https://doi.org/10.1136/bmj.308.6921.107
25. Almström M, Svensson JF, Svenningsson A, Hagel E, Wester T (2018) Population-based cohort study on the epidemiology of acute appendicitis in children in Sweden in 1987–2013. BJS Open 2(3):142–150. https://doi.org/10.1002/bjs5.52
26. Coward S, Kareemi H, Clement F et al (2016) Incidence of appendicitis over time: a comparative analysis of an administrative healthcare database and a pathology-proven appendicitis registry. PLoS ONE 11(11):e0165161. https://doi.org/10.1371/journal.pone.0165161

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Second Publication

diagnostics

MDPI

Article

Serum and Urine Biomarker Leucine-Rich Alpha-2 Glycoprotein 1 Differentiates Pediatric Acute Complicated and Uncomplicated Appendicitis

Mohit Kakar [1,2,*], Marisa Maija Berezovska [1,2], Renars Broks [3], Lasma Asare [4], Mathilde Delorme [5], Emile Crouzen [5], Astra Zviedre [1,2], Aigars Reinis [3], Arnis Engelis [1,2], Juta Kroica [3], Amulya Saxena [6] and Aigars Petersons [1,2]

[1] Department of Pediatric Surgery, Children's Clinical University Hospital, LV-1004 Riga, Latvia; marisamaija@gmail.com (M.M.B.); astra.zviedre@rsu.lv (A.Z.); arnis.engelis@rsu.lv (A.E.); aigars.petersons@rsu.lv (A.P.)
[2] Department of Pediatric Surgery, Riga Stradins University, LV-1007 Riga, Latvia
[3] Department of Biology and Microbiology, Riga Stradins University, LV-1007 Riga, Latvia; renars.broks@rsu.lv (R.B.); aigars.reinis@rsu.lv (A.R.); juta.kroica@rsu.lv (J.K.)
[4] Statistical Unit, Riga Stradins University, LV-1007 Riga, Latvia; lasma.asare@rsu.lv
[5] Faculty of Medicine, Riga Stradins University, LV-1007 Riga, Latvia; mathildedelorme16@gmail.com (M.D.); emilecrouzen@gmail.com (E.C.)
[6] Department of Pediatric Surgery, Chelsea Children's Hospital, Chelsea and Westminster NHS Fdn Trust, Imperial College London, London SW10 9NH, UK; Amulyasaxena@gmail.com
* Correspondence: mohitez@gmail.com; Tel.: +371-2876-9660

check for updates

Citation: Kakar, M.; Berezovska, M.M.; Broks, R.; Asare, L.; Delorme, M.; Crouzen, E.; Zviedre, A.; Reinis, A.; Engelis, A.; Kroica, J.; et al. 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

Academic Editor: Bogdan Sorea

Received: 14 April 2021
Accepted: 7 May 2021
Published: 11 May 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract: Purpose: This prospective, single-center cohort study analyzes the potential of inflammatory protein mediator leucine-rich alpha-2 glycoprotein 1 (LRG1) for the early and accurate diagnosis of acute appendicitis (AA), and differentiation of acute complicated (AcA) from uncomplicated appendicitis (AuA). Methods: Participants were divided into the AcA, AuA, and control groups, and their serum (s-LRG1) and urine LRG1 (u-LRG1) levels were assayed preoperatively on the second and fifth postoperative days. Results: 153 patients participated, 97 had AA. Preoperative u-LRG1 with a cut-off value of 0.18 µg/mL generated an area under the receiver operated characteristic (AUC) curve of 0.70 (95% CI 0.62–0.79) for AA versus control ($p < 0.001$), while the results for AcA versus AuA were not significant (AUC 0.60, 95% CI 0.49–0.71, $p = 0.089$). The s-LRG1 levels of AA versus the control with a cut-off value of 51.69 µg/mL generated an AUC of 0.94 (95% CI 0.91–0.99, $p < 0.001$). The cut-off value of s-LRG1 was 84.06 µg/mL for diagnosis of AcA from AuA, and therefore, significant (AUC 0.69, 95% CI 0.59–0.80, $p = 0.001$). Conclusions: LRG1 exhibited excellent diagnostic performance as an inexpensive, non-invasive, rapid, and accurate biomarker able to reflect the pathogenesis of AA. LRG1 has the potential to replace advanced imaging to diagnose clinically ambiguous AA cases.

Keywords: pediatric appendicitis; biomarkers; serum LRG1; urine LRG1

1. Introduction

Acute appendicitis (AA) is the most common pediatric surgical emergency, which may result in abscess, peritonitis, sepsis, ileus or death due to delayed diagnosis and treatment [1–3]. AA diagnosis currently depends on typical clinical findings and anamnestic evidence despite the advancement of various diagnostic techniques. The recent interest and evidence of non-surgical treatment with antibiotic therapy leads to the recurrent issue of differentiating acute uncomplicated appendicitis (AuA) from acute complicated appendicitis (AcA) upon presentation in the emergency department. Swift confirmation of appendicitis is impeded by a variety of factors such as atypical presentation and multiple differential diagnoses, thus elevating the likelihood of complications. In Latvia, delayed diagnosis is caused by perpetuating diagnostic steps in more than 35% of the cases [4].

The Alvarado score is a frequently utilized tool for AA symptom gradation, yet it lacks specificity and sensitivity [5]. Although appendicitis is particularly common in children, the clinical history assessment is often marred by the lack of cooperation and fluency [6–10]. To promote accuracy, evaluation methods such as a computed tomography (CT) and diagnostic laparoscopy are applied, but nevertheless these are time inefficient, costly, and invasive (e.g., CT-radiation increasing the long-term cancer risk) [9]. Current diagnostics such as leukocytosis, increased serum C-reactive protein (CRP), and abdominal ultrasound (US) imaging have assisted in decreasing the frequency of diagnostic laparoscopies [10]. Despite these diagnostic tools, current negative appendectomy rates of 4–45% have stimulated the search for a non-invasive strategy to lower these error figures, which has led to a rise of novel biomarkers being introduced for both the assessment and detection of appendicitis [1,11].

Multiple studies have revealed the success of the efficient diagnostic scheme provided by inflammatory biomarkers as a non-invasive analysis, increasing accuracy and speed of diagnosis, and reducing healthcare costs dramatically [12]. As immunological pathways are better understood, more biomarkers have been proposed as potential diagnostic tools, however, none are in widespread use. In comparison to Interleukin-6 (IL-6) and CRP, no other biomarkers have been proven effective in diagnosing appendicitis. The prospect of a biomarker with an even higher accuracy rate than those currently investigated is galvanizing.

Leucine-rich Alpha-2 Glycoprotein (LRG1) is a novel biomarker and is hypothesized to not only have a particularly vital and rapid diagnostic precision ratio, but also can determine specificity in acute appendicitis development with drug-independent serum-values [7,13,14]. Although its complete mechanism of action is still unclear, LRG is thought to play a role in the activation and chemotaxis of neutrophils as they enter areas of inflammation [13,14]. LRG-1 is a 50 kD membrane-associated acute phase protein of the Leucine-rich Repeat (LRR) motif, consisting of 312 amino acids— 66 of which are leucine [14,15]. LRG-1 is produced and secreted by hepatocytes, neutrophils, macrophages, and intestinal epithelium, enabling it to not be strictly contingent on any of these cells, and is upregulated in acute phase responses of microbial infections at inflammatory sites [13–15]. Its normal serum level is hypothesized to be 21–50 µg/mL [15].

Numerous pro-inflammatory markers such as IL-6, IL-1β, IL-22, TNF-α, and lipopolysaccharides stimulate the transcription of LRG1, therefore, it is not contingent on a singular stimulating factor [15]. Another peculiar facet of LRG1 is the remarkably increased concentration at the local site of inflammation, possibly distinguishing infections based on marked LRG1 deposition [15]. The current diagnostic issues of AcA show the necessity to find new early diagnostic indicators for pediatric patients to reduce the incidence of complications. The primary study objective is to demonstrate that LRG1 could potentially differentiate AcA from AuA in a prospective cohort study with cases of pediatric acute appendicitis.

2. Materials and Methods

2.1. Study Design and Setting

A prospective, single-center, randomized controlled cohort study consisted of children admitted to the Emergency Department (ED) at the Children's Clinical University Hospital (CCUH) in Riga with suspected appendicitis. This tertiary hospital is the only facility in Latvia specialized in treating children, and more than 6000 patients annually are admitted to the ED. The study meets the basic principles of the Helsinki Declaration and the requirements of the Patient's Data Protection Law. The Ethics Committee's approval was received by both the Children's Clinical University Hospital and Riga Stradins University (reference number: SP-37/2018 and 21/27.04.2017, respectively) between January 2017 and 2020, during which the research was conducted.

2.2. Study Population

Participants of this study were children between the ages of 7 and 17 who presented to the ED with suspected appendicitis. Control group participants were patients without any suspected inflammatory process in the urinary, gastrointestinal or respiratory tracts;

therefore, the included ED admitted pediatric patients of similar ages treated for various traumas (e.g., fractures, dislocations, contusions, muscle tears, testicular torsion, blunt abdominal trauma). The exclusion criteria for suspected appendicitis patients involved prior abdominal surgery, pregnancy, and chronic medical and malignant conditions that could potentially affect the urinary, gastrointestinal, or respiratory systems (e.g., inflammatory bowel disease, chronic pancreatitis, acute kidney injury, and immunosuppressed patients). The allotment of approximately 150 patients limited the study to not include patients with non-specific abdominal pain, therefore, the focus needed to be determined whether the biomarker levels differ in appendicitis patients and patients without any gastrointestinal, urinary or respiratory tract afflictions. Patient group size was divided to be in equal amounts in order to limit the assumption of variances similar to the Levene' s test.

2.3. Study Protocol

Each patient's medical history, physical examination, Alvarado score, and biochemical blood analysis were collected by the treating physician per the hospital's protocol (Nr. REK-052/01). If a patient with suspected appendicitis received an Alvarado score of six or more, a surgical consultation was prescribed and, if necessary, additional radiological imaging was considered, such as abdominal US and/or CT. Once appendicitis was confirmed and all inclusion criteria were met, a consent form was given to the patient and his or her caregiver. This consent form contained the research objective, methodology of biological materials used, and the process of biological material collection. This same consent form was given to caregivers and patients of the control group.

All participants were screened for LRG1 levels from serum and urine specimens on the day of admission, and subsequently on the second and fifth postoperative day for AA participants. The current trend for treating pediatric appendicitis to predominately perform minimal invasive techniques for surgical treatment and therefore, laparoscopic appendectomy is more common. The patients were operated by certified pediatric surgeons of the CCUH with a co-author as an assistant or the operating surgeon. Intraoperatively, microbiological cultures were obtained from the patient's peritoneal cavity. After appendectomy, additional microbiological cultures were taken from the appendix and then the appendix was sent for further histological examination. Patients were objectively classified as AuA or AcA by the absence or presence of bacterial growth in the peritoneal cavity. The primary outcome was to determine whether there is a biomarker level difference between appendicitis and non-appendicitis patients; secondarily, whether this biomarker differentiated between AuA and AcA.

2.4. Serum LRG1 Collection and Analysis

Commercially available Human Leucine-rich alpha-2-Glycoprotein 1 ELISA kit (Catalog No. NBP2-60577, Novus Biologicals, USA) were used to determine LRG1 levels, per the manufacturer's instruction manual. The minimum amount of blood required per patient for LRG1 was 300 µL per analysis (baseline, second and fifth postoperative day). The serum was collected and stored at −80 °C after centrifugation. All of the samples were measured in three different wells with results appearing within approximately 2–4 h. This kit used the ELISA method to detect human LRG1 that employs a quantitative sandwich enzyme immunoassay technique. The patient samples were sandwiched by the immobilized antibody and pre-coated LRG1-specific polyclonal antibody wells, and subsequently recognized by the streptavidin-peroxidase conjugate. After the unbound materials were washed away, a peroxidase enzyme substrate was added from which the color intensity could be measured. The minimum detectable LRG1 serum level was 0.313 ng/mL. Obtained values in ng/mL were converted to µg/mL for further calculations.

2.5. Urine LRG-1 Collection and Analysis

The same kit and instruction manual procedure were employed for urine analyses. Midstream, clean-catch urine specimens of at least 200 µL were collected in a sterile cup on

admission, and subsequently for AA participants on the second and fifth postoperative day. Most AA patients had their day 0 urine sample collected during operation through a urine catheter. Urine samples were centrifuged at 2000 rpm for 10 min at 4 °C and then these supernatants were stored at −80 °C before analysis. The following day, the samples were transported to the laboratory for processing. The same steps of ELISA, as performed on s-LRG1, were also executed on these samples.

2.6. Statistical Analysis

Microsoft Excel 2016 and IBM SPSS Statistics 26 were utilized for statistical analyses and the data was validated by a statistical analyst for accuracy. Results were expressed as median values and interquartile ranges (IQR). The comparison between groups was calculated using the Mann–Whitney U-Test for two groups and Kruskal–Wallis test for all three groups for quantitative variables, which do not follow normal distribution. The Fisher Exact Test and Pearson Chi-square test were applied on qualitative variables to determine associations between the groups. 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; therefore, this determined the clinical importance of the biomarkers, as well as their diagnostic value regarding appendicitis. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical Characteristics of the Study Population

During the study, samples were collected from 153 patients eligible for this research; 97 were diagnosed with appendicitis and 56 had no suspected infectious or inflammatory pathology. Participant ages ranged from 7 to 17 years, with a median of 12 years; 58% of which identified as male and 42% female. Patients with AA underwent appendectomy, and intraoperative swabs of free peritoneal fluid were collected to detect potential bacterial growth in the peritoneal cavity to distinguish AcA 52 (53.6%) from AuA 45 (46.4%).

Patients were predominately operated laparoscopically (87.6%), and only four AuA and eight AcA patients were operated conventionally. Nine AuA (22.5%) and 31 AcA (77.5%) patients required the placement of a drainage tube; 83 AA patients received an abdominal ultrasound, but only 69 patients' diagnoses were confirmed for acute appendicitis. Demographics and clinical characteristics of the patients are presented in Table 1.

Table 1. Demographics and clinical characteristics of cohort population.

	AuA	AcA	Ctr	Total	*p*-Value
	n = 45	*n* = 52	*n* = 56	*n* = 153	
Gender, *n* (%)					
Boy	23 (14.4)	27 (18.3)	39 (25.5)	89 (58.2)	0.081 *
Girl	23 (15.0)	24 (15.7)	17 (11.1)	64 (41.8)	
Age, Mdn (IQR)	13.0 (10.0–15.0)	12.0 (9.0–14.0)	13.5 (10.3–15.0)	-	0.101 ***
Type of surgery, *n* (%)				85 (87.6)	
Laparoscopy	41 (91.1)	44 (84.6)	-		0.333 *
Laparotomy	4 (8.9)	8 (15.4)	-	12 (12.4)	
Ultrasound, *n* (%)	30 (43.5)	39 (56.5)	-	69 (0.83)	0.349 *
Drainage tube, *n* (%)				40	
Yes	9 (20.9)	31 (60.8)	-		**<0.001** *
No	34 (79.1)	20 (39.2)	-	54	
Length of hospital stay, days (IQR)	5 (4–6)	6 (4–9)	-	-	**0.002** **

AcA = Acute complicated appendicitis, AuA = Acute uncomplicated appendicitis, Ctr = control group. Median values are presented with IQR (25%; 75%). *—Pearson Chi-square test, **—Mann–Whitney U-test, ***—Kruskal–Wallis test.

3.2. Serum Leucine-Rich Alpha Glycoprotein-1 Levels

Baseline preoperative s-LRG1 concentrations are presented in Table 2, along with values of the second and fifth postoperative days. s-LRG1 median value is more than twice as high in AuA compared to control group and almost three times as high in AcA. The median preoperative s-LRG1 values for AcA, AuA, and the control group were 88.12 µg/mL, 70.56 µg/mL, and 34.08 µg/mL respectively. Additional assessment of the dependency between s-LRG1 concentration and disease grade in AA patients is demonstrated in Figure 1, which reveals 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 (AcA versus AuA) $p = 0.001$ when compared between AcA vs. AuA only.

Table 2. Pre- and post-operative LRG1 levels per appendicitis status.

	AcA, µg/mL (IQR)	AuA, µg/mL (IQR)	Control, µg/mL (IQR)	*p*-Value
DAY 0				
Serum	70.56 (62.64–83.43)	88.12 (71.12–106.13)	34.08 (27.50–42.37)	<0.001
Urine	0.10 (0.03–0.73)	0.35 (0.05–1.38)	0.04 (0.02–0.10)	<0.001
DAY 2				
Serum	74.99 (61.00–96.03)	87.90 (70.32–104.10)		0.048
Urine	0.08 (0.03–0.28)	0.21 (0.06–0.98)		0.017
DAY 5				
Serum	66.73 (56.98–85.28)	80.97 (62.14–99.03)		0.110
Urine	0.04 (0.02–0.27)	0.10 (0.03–0.25)		0.102

AcA = Acute complicated appendicitis, AuA = Acute uncomplicated appendicitis, LRG1 = Leucine-rich alpha glycoprotein-1. Median values are presented with IQR (25%; 75%).

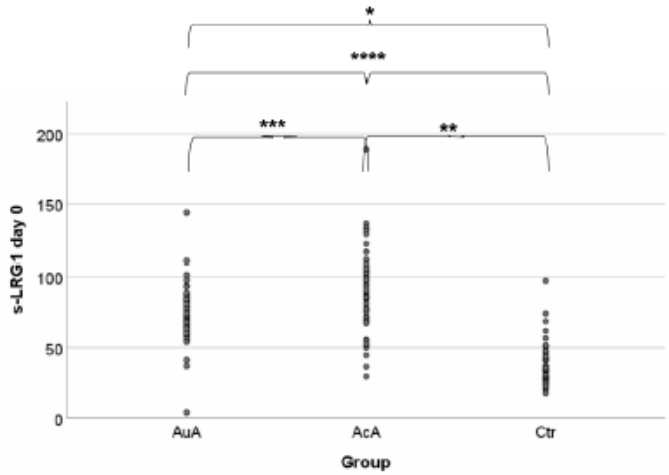


Figure 1. s-LRG1 of each patient group. s-LRG1 levels are increased in patients with AA ($n = 97$) compared to the control group ($n = 56$), **** $p < 0.001$. s-LRG1 could detect disease progress when analyzing AcA ($n = 52$) and AuA ($n = 45$), *** $p = 0.001$. A significant difference was recognized when the control group was compared to AcA and AuA separately; * $p < 0.001$ and ** $p < 0.001$, respectively.

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 ED admission ($p < 0.001$) (Table 2). Thus, these results suggest s-LRG1, as a novel biomarker after appendectomy, correlates with patient recovery.

3.3. Urine Leucine-Rich Alpha-2 Glycoprotein-1 Levels

Baseline preoperative values and values on the second and fifth postoperative days of u-LRG1 are shown in Table 2. The median preoperative u-LRG1 levels for AcA, AuA, and control group were 0.35 µg/mL, 0.10 µg/mL, and 0.04 µg/mL respectively; therefore, the control group presented with the lowest baseline level. Further assessment of whether u-LRG1 levels were associated with disease activity in patients with AA is demonstrated in Figure 2. This reveals 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 ($p = 0.089$).

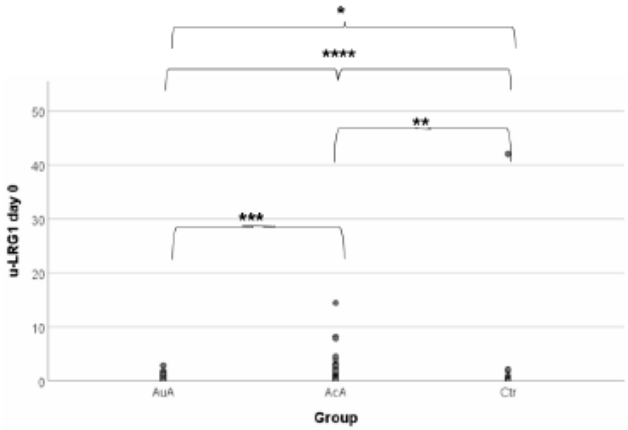


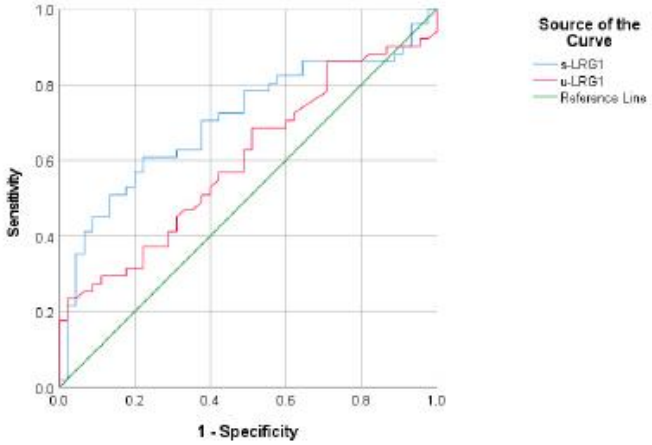
Figure 2. u-LRG1 of each patient group. u-LRG1 levels are increased in patients with AA ($n = 97$) compared to the control group ($n = 56$), **** $p < 0.001$. u-LRG1 was not able to detect disease progress when analyzing AcA ($n = 52$) and AuA ($n = 45$), *** $p = 0.089$. A significant difference was recognized when the control group was compared to AcA and AuA separately; * $p < 0.001$ and ** $p = 0.005$, respectively.

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, ($p < 0.001$) (Table 2). u-LRG1 concentrations dropped by more than 50% (52/93 patients) after the diseased appendix was resected. Thus, these results suggest u-LRG1, as a novel biomarker, correlates with the improvement of patient recovery post-appendectomy.

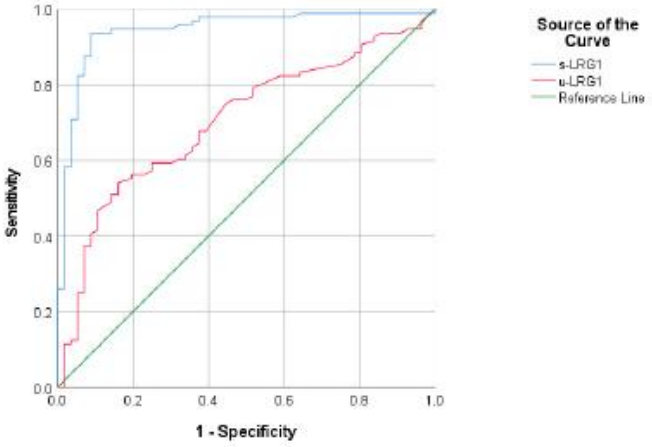
3.4. Threshold Sensitivity and Specificity of Leucine-Rich Alpha-2 Glycoprotein-1

Given the potential role of LRG1 as a biomarker for clinical diagnosis of AA, additional investigation of its diagnostic accuracy in detecting the complicated course of disease was performed by analyzing sensitivity and specificity of serum and urine LRG1 through the application of ROC curves and AUC analysis. The s-LRG1 cut-off value for patients with AA was 51.69 µg/mL, and for AcA was 84.05 µg/mL. The ROC curves demonstrate an

AUC of 0.95 (95% CI 0.91–0.99) and 0.69 (95% CI 0.59–0.80) respectively (Figure 3A,B). s-LRG1 in AA had 93.8% sensitivity and 91.1% specificity, while in AcA, it displays a lower sensitivity and specificity: 59.6% and 77.8%.



(**A**)



(**B**)

Figure 3. Analysis of s-LRG1 and u-LRG1 for sensitivity and specificity. The ROC curve for s-LRG1 analysis AcA vs. AuA children demonstrated AUC of 0.69 (95% CI 0.59–0.80) at cut-off value of 84.05 µg/mL and for u-LRG1 AUC of 0.60 (95% CI 0.49–0.71): (**A**) The ROC curve for s-LRG1 analysis AA vs. Ctr demonstrates AUC of 0.95 (95% CI 0.91–0.99) at cut-off value of 51.69 µg/mL and for u-LRG1 AUC of 0.70 (95% CI 0.62–0.79) at cut-off value of 0.175 µg/mL (**B**).

The u-LRG1 cut-off value for AA patients was 0.175 µg/mL, and for AcA was not significant. The ROC curves demonstrate an AUC of 0.70 (95% CI 0.62–0.79) and 0.60 (95% CI 0.49–0.71) respectively (Figure 3A,B). u-LRG1 for appendicitis had a sensitivity of 54.2% and specificity of 83.9%. The efficiency of u-LRG1 as a predictive marker of AcA versus AuA was found to be insignificant ($p = 0.089$) (Figure 3A). Contrarily, u-LRG1 proves to be an adequate predictive marker of AA ($p < 0.001$) (Figure 3B).

4. Discussion

Accurate, early diagnosis of acute appendicitis continues to be a major diagnostic challenge, and biomarkers potentially can confirm appendicitis and determine the severity. Current inflammatory markers of leukocytosis and CRP are too vague to accurately determine appendicitis with high specificity or sensitivity [5]. The authors of this study assessed LRG1 as a potential diagnostic tool because its marked expression is believed to represent an increased inflammatory process in patients with appendicitis. The primary objective was to evaluate the significant potential of LRG1 in distinguishing AcA from AuA as to commence adequate therapy. This study demonstrated that patients with appendicitis had significantly elevated s-LRG1 and u-LRG1 concentrations compared to the control group and had a significant difference of s-LRG1 concentrations in AcA and AuA, indicating that s-LRG1 correlates with the severity of appendicitis.

This study met many limitations as testing was performed at a single-center with a finite range of resources during inconsistent hours which limited patient participation. Most patients received either antibiotic and/or intravenous fluid therapy prior to surgery and sample collection, which may have affected biomarker concentrations. The usage of different Human Leucine-rich alpha-2-Glycoprotein 1 ELISA kits by other publications make the comparison with the results of this study arduous. The analysis of LRG1 samples was performed at a clinical research laboratory, not at the hospital's laboratory, and therefore, concentrations could have been affected. The required duration to process urine and serum LRG1 in a clinical care setting has been reported to be two hours by Salö et al. with the new generation 'Ultrafast ELISA Assay' [16]. Patient quota limited the multiple types of patients that could be included; however, this study provided a basis for future broader studies, which are already underway. Patients under seven were excluded—as that age range tends to have a different pathophysiology for appendicitis as there is a strong link to bouts of viral infections such as gastroenteritis—and pre-hospitalization anamnestic information can be quite limited. The authors already have started another research study which includes these young patients.

A diagnosis of appendicitis needs to be ruled out from non-specific abdominal pain, and quite frequently, laboratory analyses and a surgical consult are the tools. Patients with non-surgical abdominal pain were not included in this study due to the patient quota. It was important to determine whether there was any basis for expanding this type of study regarding LRG1 diagnostic abilities in acute appendicitis. Multiple recent publications demonstrate the correlation between an enriched s-LRG1 concentration and the confirmed diagnosis of AA in children, which also is supported by this study's findings [7,12–14,17–19]. A vast amount of clinical reports observes increased s-LRG1 levels in the development of a variety of disorders such as inflammatory disorders (intestinal, renal, and respiratory systems), oncological pathology (e.g., colorectal cancer, hepatocellular carcinoma, pancreatic cancer, ovarian cancer, lung cancer) and other chronic conditions (e.g., ulcerative colitis (UC), hydrocephalus, heart failure) [15,20–29]. The presence of LRG1 in diseased appendices and serum can be explained by neutrophils secreting LRG1 in response to bacteria [12,14,19,25,30]. Its role in inhibiting cell apoptosis by binding to Cytochrome C stimulates lymphocyte survival in the appendix and protects appendiceal tissue from susceptibility to toxicity [31]. In addition, LRG1 binds to accessory receptors of Transforming Growth Factor-β regulating a signaling pathway that stimulates angiogenesis, which may enhance tissue inflammation [25,30]. These studies also did not include patients with non-specific abdominal pain and therefore, further investigation is warranted.

Yap et al. revealed the efficient combination of s-LRG1 analysis with Alvarado score assessment had improved diagnostic accuracy of AA, creating the possibility of diagnosing AA at the ED without radiological confirmation [19]. The data show that s-LRG1 has markedly high specificity and sensitivity for the confirmation of suspected AA (Figure 3B), which leads to agreement with the suggested strategy of Yap et al. to replace IL-6 with s-LRG1 in the Alvarado score with a proposed lower limit of 51.69 µg/mL as a standard for suspect AA. Anupam et al. produced a cut-off value of 40.15 µg/mL [30].

Time is a vital factor in the progression of AA. Subacute appendicitis cannot be detected by CRP, IL-6 or leukocytes. Neutrophils act as first responders to infection, which explains the rapid detection of AA by LRG1, as it is secreted by neutrophils. Additionally, LRG1 has a longer half-life than CRP, thus the time range in AA is in favor of LRG1 [15]. CRP production is primarily dependent on liver stimulation by IL-6, whereas LRG1 is secreted by hepatocytes, intestinal epithelium, neutrophils, and macrophages if induced by IL-22, IL-1β, TNF-α and LPS apart from IL-6 [13–15,30]. Markedly high s-LRG1 is immediately associated with high vulnerability to a complicated appendicitis and hence is indicated for emergency appendectomy. Elevation of s-LRG1, to an ambiguous extent with low potential of AcA, grants valid grounds for antibiotic therapy. It was demonstrated in this study that s-LRG1 levels lowered in response to treatment in patients' post-appendectomy and receiving antibiotic therapy. To evaluate positive treatment response, s-LRG1 can be re-evaluated on second and fifth day of antibiotic therapy to exclude the necessity of invasive treatment. This proposal may aid future approach to conservative AA treatment strategy.

u-LRG1 levels of pediatric appendicitis patients were significantly elevated in comparison to the control group in this study (0.10–0.35 µg/mL and 0.04 µg/mL respectively, $p < 0.001$). This confirms previously published statements on LRG1 as an accurate diagnostic method, superior to current urinary inflammatory markers [13,14,16,17,32]. From Figure 3, the authors deduct that starting from a u-LRG1 value of 0.175 µg/mL could indicate AA. Anupam et al. generated a cut-off value of 0.042 µg/mL using an alternative ELISA [30].

The renal threshold and selective filtering of LRG1 are unknown, but its marked elevation in urine proposes its local release by inflammatory sites (e.g., mesoappendix) and/or neutrophils [12,14,33]. Lee et al. explained LRG1 expression in urine by analyzing renal tubular injury caused by proteinuria, which induces an NLRP3 activation and maturation of IL-1β, and therefore, results in a positive stimulation of LRG1 in tubular epithelial cells in various renal disorders [22]. It is necessary to investigate more on the physiology of LRG1 in the kidney as it is increased in AA cases, which typically do not have renal impairment. Rodriguez-Suarez et al. found that, beside the merit of LRG1 to AA diagnosis, their results of increased u-LRG1 levels corresponded to the histological severity of AA [32]. When they applied Selected Reaction Monitoring (SRM), their results demonstrated that u-LRG1 is elevated 100-fold in patients with acute appendicitis compared to those without [32]. This study's results with ELISA merely showed a mild significant difference between AA and control, and thus, when comparing AcA and AuA, there was no sufficient difference (0.42 and 0.33 µg/mL respectively, $p = 1.000$).

The physiological characteristics of LRG1 include the ability to distinguish AA severity, its rapid response to inflammation, resolute accuracy, enduring half-life, and independence from stimulatory and secretory mechanisms. Therefore, LRG1 qualifies as a more adequate and efficient biomarker compared to those currently applied. It should be emphasized that the results of the study in Latvia could improve the quality of medical care in relatively low socio-economic situated countries. Thus, urinalysis of LRG1 as an inexpensive, non-invasive diagnostic tool that could substitute imaging techniques, altering present diagnostic guidelines. However, prospective studies in large populations should consider other inflammatory markers so as to increase diagnostic efficacy of severity of appendicitis. The accuracy of u-LRG1 and s-LRG1 in diagnosing AA should be more elaborately investigated, focusing on its pathophysiology (e.g., in the renal and intestinal tract). LRG1

deserves a wider research scope in various institutions to continue its verification as a valuable diagnostic tool in AA; the approach of which must involve the exclusive use of one type of Human Leucine-rich alpha-2-Glycoprotein 1 ELISA kit. The response of LRG1 to antibiotic treatment promises interesting possibilities of altering therapy evaluation. Additional investigations remain to be completed regarding the response of LRG1 to diverse types of bacteria and different classes of antibiotic and intravenous fluid therapy. The fact that s-LRG1 is significantly increased in AcA when compared to AuA, enables speculation of its spread to the peritoneal fluid, which, thus far, is insufficiently examined. Cut-off values of s-LRG1 or u-LRG1 for the determination of AA or AcA need to be investigated further so a standard of diagnostic LRG1 values may be confirmed. Furthermore, the potential of LRG1 mRNA as an accurate marker for AA should be investigated.

5. Conclusions

The results suggest that along with clinical suspicion of AA, LRG1 is an accurate marker in diagnosis confirmation. The severity of AA with respect to AcA and AuA can be distinguished by the s-LRG1 concentration, and therefore, the evaluation of s-LRG1 could prove useful to initiate adequate therapy and analysis of disease status. u-LRG1 and s-LRG1 exhibited excellent diagnostic performances as inexpensive, non-invasive, rapid, and accurate biomarkers that reflect the pathogenesis of AA. The results of this study could improve the quality of medical care in relatively low socio-economic countries.

Author Contributions: Study conception and design were developed by M.K., A.E., A.P., J.K., A.R., A.S., A.Z. and M.M.B. Data acquisition was carried out by M.K., M.M.B. and R.B. Analyses and data interpretation were performed by M.K., R.B., M.D., E.C., L.A. and J.K. This article's manuscript was drafted by M.K., M.M.B., M.D. and E.C. Critical revision was executed by all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This research received grant support from the Latvian Council of Science and Riga's Stradins University. Grant support was used for the laboratory materials for biological specimen collection.

Institutional Review Board Statement: The study was conducted according to guidelines of the Declaration of Helsinki. The Ethics Committee's approval was received by both the Children's Clinical University Hospital and Riga Stradins University (reference number: SP-37/2018 and 21/27.04.2017, respectively) between January 2017 and 2020, during which the research was conducted.

Informed Consent Statement: Written informed consent was obtained from all subjects and their parents involved in the study.

Acknowledgments: The authors gratefully acknowledge the nurses and staff of the Children's Clinical University Hospital in Riga and the commitment of participating patients for their invaluable contribution.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AA	Appendicitis
AcA	Acute complicated appendicitis
AuA	Acute uncomplicated appendicitis
ELISA	Enzyme-linked immunosorbent assay
IL-6	Interleukin 6
s-LRG1	Serum Leucine-rich alpha-2 glycoprotein 1
TNF-α	Tumor necrosis factor-alpha
u-LRG1	Urine Leucine-rich alpha-2 glycoprotein 1

References

1. Joshi, M.K.; Joshi, R.; Alam, S.E.; Agarwal, S.; Kumar, S. Negative Appendectomy: An Audit of Resident-Performed Surgery. How Can Its Incidence Be Minimized? *Indian J. Surg.* **2015**, *77*, 913–917. [CrossRef]
2. D'Souza, N.; Nugent, K. Appendicitis. *BMJ Clin. Evid.* **2014**, *8*, 0408.

3. Blomqvist, P.G.; Andersson, R.E.B.; Granath, F.; Lambe, M.P.; Ekbom, A.R. Mortality after appendectomy in Sweden, 1987–1996. *Ann. Surg.* **2001**, *233*, 455–460. [CrossRef] [PubMed]
4. Zviedre, A.; Engelis, A.; Tretjakovs, P.; Jurka, A.; Zile, I.; Petersons, A. Role of serum cytokines in acute appendicitis and acute mesenteric lymphadenitis among children. *Medicina* **2016**, *52*, 291–297. [CrossRef] [PubMed]
5. Podany, A.B. Acute Appendicitis in Pediatric Patients: An Updated Narrative Review. *J. Clin. Gastroenterol. Treat.* **2017**, *3*, 1–9. [CrossRef]
6. Kingsnorth, A.; Reilly, D.O. Clinical review Acute pancreatitis. *BMJ* **2006**, *332*, 1072–1076. [CrossRef] [PubMed]
7. Kharbanda, A.B.; Rai, A.J.; Cosme, Y.; Liu, K.; Dayan, P.S. Novel serum and urine markers for pediatric appendicitis. *Acad. Emerg. Med.* **2012**, *19*, 56–62. [CrossRef] [PubMed]
8. Almaramhy, H.H. Acute appendicitis in young children less than 5 years: Review article. *Ital. J Pediatr.* **2017**, *43*, 1–9. [CrossRef]
9. Wray, C.J.; Kao, L.S.; Millas, S.G.; Tsao, K.; Ko, T.C. Acute Appendicitis: Controversies in Diagnosis and Management. *Curr. Probl. Surg.* **2013**, *50*, 54–86. [CrossRef]
10. Coursey, C.A.; Nelson, R.C.; Patel, M.B.; Cochran, C.; Dodd, L.G.; DeLong, D.M.; Vaslef, S. Making the diagnosis of acute appendicitis: Do more preoperative CT scans mean fewer negative appendectomies? A 10-year study. *Radiology* **2010**, *254*, 460–468. [CrossRef]
11. Bachur, R.G.; Hennelly, K.; Callahan, M.J.; Chen, C.; Monuteaux, M.C. Diagnostic imaging and negative appendectomy rates in children: Effects of age and gender. *Pediatrics* **2012**, *129*, 877–884. [CrossRef]
12. Kentsis, A.; Lin, Y.Y.; Kurek, K.; Calicchio, M.; Wang, Y.Y.; Monigatti, F.; Bachur, R. Discovery and Validation of Urine Markers of Acute Pediatric Appendicitis Using High-Accuracy Mass Spectrometry. *Ann. Emerg. Med.* **2010**, 55. [CrossRef]
13. Kentsis, A.; Ahmed, S.; Kurek, K.; Brennan, E.; Bradwin, G.; Steen, H.; Bachur, R. Detection and diagnostic value of urine leucine-rich α-2-glycoprotein in children with suspected acute appendicitis. *Ann. Emerg. Med.* **2012**, *60*, 78–83. [CrossRef]
14. Naka, T.; Fujimoto, M. LRG is a novel inflammatory marker clinically useful for the evaluation of disease activity in rheumatoid arthritis and inflammatory bowel disease. *Immunol. Med.* **2018**, *41*, 62–67. [CrossRef]
15. Serada, S.; Fujimoto, M.; Terabe, F.; Iijima, H.; Shinzaki, S.; Matsuzaki, S.; Naka, T. Serum leucine-rich alpha-2 glycoprotein is a disease activity biomarker in ulcerative colitis. *Inflamm. Bowel. Dis.* **2012**, *18*, 2169–2179. [CrossRef] [PubMed]
16. Kentsis, A. Challenges and opportunities for discovery of disease biomarkers using urine proteomics. *Pediatr. Int.* **2011**, *53*, 1–6. [CrossRef] [PubMed]
17. Druhan, L.J.; Lance, A.; Li, S.; Price, A.E.; Emerson, J.T.; Baxter, S.A.; Avalos, B.R. Leucine rich α-2 glycoprotein: A novel neutrophil granule protein and modulator of myelopoiesis. *PLoS ONE* **2017**, *12*, e0170261. [CrossRef] [PubMed]
18. Zhang, Q.; Huang, R.; Tang, Q.; Yu, Y.; Huang, Q.; Chen, Y.; Wang, X. Leucine-rich alpha-2-glycoprotein-1 is up-regulated in colorectal cancer and is a tumor promoter. *Oncotargets Ther.* **2018**, *11*, 2745–2752. [CrossRef]
19. Oikonomou, K.A.; Kapsoritakis, A.N.; Theodoridou, C.; Karangelis, D.; Germenis, A.; Stefanidis, I.; Potamianos, S.P. Neutrophil gelatinase-associated lipocalin (NGAL) in inflammatory bowel disease: Association with pathophysiology of inflammation, established markers, and disease activity. *J. Gastroenterol.* **2012**, *47*, 519–530. [CrossRef]
20. Shinzaki, S.; Matsuoka, K.; Iijima, H.; Mizuno, S.; Serada, S.; Fujimoto, M.; Naka, T. Leucine-rich Alpha-2 glycoprotein is a serum biomarker of mucosal healing in ulcerative colitis. *J. Crohn's Colitis* **2017**, *11*, 84–91. [CrossRef] [PubMed]
21. O'Donnell, L.C.; Druhan, L.J.; Avalos, B.R. Molecular characterization and expression analysis of leucine rich alpha2-glycoprotein, a novel marker of granulocytic differentiation. *J. Leukoc. Biol.* **2002**, *72*, 478–485.
22. Wang, C.H.; Li, M.; Liu, L.L.; Zhou, R.Y.; Fu, J.; Zhang, C.Z.; Yun, J.P. LRG1 expression indicates unfavorable clinical outcome in hepatocellular carcinoma. *Oncotarget* **2015**, *6*, 42118–42129. [CrossRef]
23. Andersen, J.D.; Boylan, K.L.M.; Jemmerson, R.; Geller, M.A.; Misemer, B.; Harrington, K.M.; Skubitz, A.P.N. Leucine-rich alpha-2-glycoprotein-1 is upregulated in sera and tumors of ovarian cancer patients. *J. Ovarian Res.* **2010**, *3*, 21. [CrossRef]
24. Zhang, J.; Zhu, L.; Fang, J.; Ge, Z.; Li, X. LRG1 modulates epithelial-mesenchymal transition and angiogenesis in colorectal cancer via HIF-1α activation. *J. Exp. Clin. Cancer Res.* **2016**, *35*, 29. [CrossRef]
25. Hao, L.; Xie, H.; Zhang, B.; Chen, D.; Wang, S.; Zhang, H.; He, S. LRG1 downregulation in allergic airway disorders and its expression in peripheral blood and tissue cells. *J. Transl. Med.* **2016**, *14*, 202. [CrossRef] [PubMed]
26. Codina, R.; Vanasse, A.; Kelekar, A.; Vezys, V.; Jemmerson, R. Cytochrome c-induced lymphocyte death from the outside in: Inhibition by serum leucine-rich alpha-2-glycoprotein-1. *Apoptosis* **2010**, *15*, 139–152. [CrossRef] [PubMed]
27. Bozkurt, S.; Köse, A.; Erdogan, S.; Bozali, G.L.; Ayrik, C.; Arpaci, R.B.; Türkmenoglu, Ö. MPV and other inflammatory markers in diagnosing acute appendicitis. *J. Pak. Med. Assoc.* **2015**, *65*, 637–641.
28. Sandanayake, N.S.; Sinclair, J.; Andreola, F.; Chapman, M.H.; Xue, A.; Webster, G.J.; Pereira, S.P. A combination of serum leucine-rich α-2-glycoprotein 1, CA19-9 and interleukin-6 differentiate biliary tract cancer from benign biliary strictures. *Br. J. Cancer* **2011**, *105*, 1370–1378. [CrossRef] [PubMed]
29. Yap, T.L.; Fan, J.D.; Chen, Y.; Ho, M.F.; Choo, C.S.; Allen, J.; Nah, S.A. A novel noninvasive appendicitis score with a urine biomarker. *J. Pediatr. Surg.* **2019**, *54*, 91–96. [CrossRef]
30. Kakar, M.; Delmore, M.; Broks, R.; Asare, L.; Butnere, M.; Saxena, A.; Engelis, A.; Kroica, J.; Saxena, A.; Petersons, A. Determining Acute Complicated and Uncomplicated Appendicitis Using Serum and Urine Biomarkers: Interleukin-6 and Neutrophil Gelatinase Lipocal. *Pediatr. Surg. Int.* **2020**, *36*, 629–636. [CrossRef]

31. Rainer, T.H.; Leung, L.Y.; Chan, C.P.Y.; Leung, Y.K.; Cheng, N.M.; Lai, P.B.S.; Graham, C.A. Circulating human leucine-rich α-2-glycoprotein 1 mRNA and protein levels to detect acute appendicitis in patients with acute abdominal pain. *Clin. Biochem.* **2017**, *50*, 485–490. [CrossRef] [PubMed]
32. Saloö, M.; Roth, B.; Stenstroöm, P.; Arnbjörnsson, E.; Ohlsson, B. Urinary biomarkers in pediatric appendicitis. *Pediatr. Surg. Int.* **2016**, *32*, 795–804. [CrossRef] [PubMed]
33. Lee, H.; Fujimoto, M.; Ohkawara, T.; Honda, H.; Serada, S.; Terada, Y.; Naka, T. Leucine rich α-2 glycoprotein is a potential urinary biomarker for renal tubular injury. *Biochem. Biophys. Res. Commun.* **2018**, *498*, 1045–1051. [CrossRef] [PubMed]

Third Publication

medicina

MDPI

Article

Microbiota Assessment of Pediatric Simple and Complex Acute Appendicitis

Mohit Kakar [1,2,*], Aigars Reinis [3], Juta Kroica [3], Arnis Engelis [1,2], Renars Broks [3], Lasma Asare [4], Marelize Vermeulen [5], Simone Oliver Senica [5], Amulya Saxena [6] and Aigars Petersons [1,2]

1 Department of Pediatric Surgery, Children's Clinical University Hospital, LV-1004 Riga, Latvia
2 Department of Pediatric Surgery, Riga Stradins University, LV-1007 Riga, Latvia
3 Department of Biology and Microbiology, Riga Stradins University, LV-1007 Riga, Latvia
4 Statistics Unit, Riga Stradins University, LV-1046 Riga, Latvia
5 Faculty of Medicine, Riga Stradins University, LV-1007 Riga, Latvia
6 Department of Pediatric Surgery, Chelsea Children's Hospital, Chelsea and Westminster NHS Fdn Trust, Imperial College London, London SW10 9NH, UK
* Correspondence: mohitez@gmail.com

Abstract: Background and Objectives. The aim of this study is to determine the prevailing microbiota in samples from pediatric patients with acute appendicitis, as well as evaluate the antibacterial sensitivity of the isolated microorganisms, comparing the data obtained with the clinic's antibacterial therapy guidelines. **Materials and Methods.** The study group consisted of 93 patients between the ages of 7 and 18. All patients underwent a laparoscopic or conventional appendectomy. The children were hospitalized with signs and symptoms suggestive of acute appendicitis. Microbiological cultures from the appendix and abdominal cavity were collected intraoperatively. **Results.** *E. coli* was identified in most cases irrespective of the clinical presentation of acute appendicitis. Most strains were susceptible to ampicillin and amoxicillin/clavulanic acid. Five strains of *E. coli* produced extended spectrum beta-lactamase (ESBL). *Pseudomonas aeruginosa* (*P. aeruginosa*) was the second most commonly isolated causative agent. Furthermore, it was common in cases of acute complex appendicitis. Most strains of *P. aeruginosa* were resistant to amoxicillin/clavulanic acid, ertapenem, ampicillin and cefotaxime, yet were susceptible to ceftazidime. Regardless of the clinical presentation, the samples yielded mixed isolates. **Conclusion.** *E. coli* is the main causative agent of acute appendicitis in the pediatric population displaying susceptibility to various antibiotics. *P. aeruginosa* was more prevalent in cases of acute complex appendicitis. *P. aeruginosa* isolates were susceptible to ceftazidime; however, they were resistant to cefotaxime, which should, therefore, be removed from guidelines for empirical antibacterial treatment of acute appendicitis due to phenotypic resistance of *P. aeruginosa*. We recommend antibiotics with distinct implementation to avoid antibiotic resistance.

Keywords: simple and complex pediatric appendicitis; microbiota; *P. aeruginosa*; empirical antimicrobial treatment; antibacterial susceptibility

check for updates

Citation: Kakar, M.; Reinis, A.; Kroica, J.; Engelis, A.; Broks, R.; Asare, L.; Vermeulen, M.; Senica, S.O.; Saxena, A.; Petersons, A. Microbiota Assessment of Pediatric Simple and Complex Acute Appendicitis. *Medicina* **2022**, *58*, 1144. https://doi.org/10.3390/medicina58091144

Academic Editor: Johannes Mayr

Received: 20 July 2022
Accepted: 16 August 2022
Published: 23 August 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The therapeutic plan for acute appendicitis has advanced in children to favor non-surgical antibacterial treatment over surgical treatment. Complicated appendicitis is the most common cause of intra-abdominal infections in children [1]. In order to decrease the risk of post-operative complications in cases of complicated appendicitis, such as wound infections and intraabdominal abscesses, antibiotics are included in the treatment protocols. However, there is a lack of unity with regards to the optimum choice for antibiotic regimens in cases of acute appendicitis in children. Moreover, the most suitable regimen can be subject to change depending on the geographical distribution of species of pathogenic and opportunistic pathogens as well as their antibacterial resistance. Thus, it is important

to assess the etiopathology of pediatric appendicitis (simple and complex) clinically and analyze the antibacterial susceptibility of its causative agents [2,3].

The aim of this study is to determine the prevailing microbiota in samples from pediatric patients with acute appendicitis, as well as evaluate the antibacterial sensitivity of the isolated microorganisms, comparing the data obtained with the clinic's antibacterial therapy guidelines. The results of the study showed that *P. aeruginosa* was more common in cases of acute complex appendicitis than in acute simple appendicitis. It was resistant to cefotaxime, which should therefore not be recommended for empirical antibacterial treatment of acute appendicitis. Antibiotics with different mechanisms of action should be used for treatment of acute complex appendicitis to avoid the development of antibiotic resistance.

2. Materials and Methods

A total of 93 patients (47 males, 46 females) between the ages of 7 to 18 were enrolled for this study due to limitations with the ethics approval. Patients were admitted to the Children's Clinical University Hospital with complaints of acute abdominal pain in conjunction with signs and symptoms suggestive of appendicitis. Patients were screened pre-operatively to confirm or exclude this diagnosis. All patients underwent a laparoscopic or conventional appendectomy. Microbiological culture swabs from the appendix and peritoneal cavity were collected intraoperatively. The pediatric surgery team supervising the patients with appendicitis received written consent forms from the respective caregivers and assent from the patients if they were 13 years of age or older. The consent and assent were concerning to the research objective and methodology used for investigating the biological material [4,5]. Ethical approval: All procedures executed in this study involving human contenders were in accordance with the ethical standards of the institutional and/or the national research committee (Riga Stradins University, reference number: 21/27.04.2017.; as well as with the Children's Clinical University Hospital, reference number: SP-37/2018.), the 1975 Helsinki declaration and its amendments were also included or other comparable standards of ethics. Data including patients' age, sex, and medical history were poised prior to surgery.

Immediately following the appendectomy, the appendix was anatomized under barren circumstances, paired swab samples were taken from the intraluminal side of the appendix and an extra swab sample was taken from the submucosa. Specimens were placed in Amies transport medium for immediate transfer and subsequent bacterial culture [6]. They were cultured under aerobic and anaerobic conditions. Cultivation was performed on blood agar (Supplement, Oxoid, Hampshire, UK; Defibrinated Sheep blood—E&O laboratories limited, Falkirk, Scotland), MacConkey (Oxoid, UK) and trypticase soy (Oxid, UK) agar. Bacterial recognition was performed using the VITEK2 analyzer (Biomerieux, Auvergne-Rhone-Alpes, France).

Tests were conducted on antibacterial susceptibility, evaluations on the subsequent results were in accordance with recommendations from the European Committee on Antimicrobial Susceptibility Testing (EUCAST), more specifically 'Clinical breakpoints and dosing of antibiotics' (Version 8.0, 2020) [7]. Cultures that were cultivated overnight were suspended in physiological saline of up to 0.5 McFarland units (McFarland Densitometer DEN-1, Biosan, Riga, Latvia). The inoculation of the suspension was on Mueller-Hinton agar (Oxid, UK). Selected antibiotics were placed on the inoculated plates and included ceftazidime 10 µg, ampicillin 10 µg, cefotaxime 5 µg, meropenem 10 µg, imipenem 10 µg, amikacin 30 µg, gentamicin 10 µg, ciprofloxacin 5 µg, chloramphenicol 30 µg ertapenem 10 µg, amoxicillin/clavulanic acid 30 µg and piperacillin/tazobactam 36 µg (Liofilchem, Roseto degli Abruzzi, Italy). Plates were inoculated at a temperature of +35 ± 1 °C for 18 ± 2 h. According to the EUCAST standard, the double-disk synergy test (DDST) was used to detect extended spectrum beta-lactamase *E. coli* (ESBL). Results were assessed by measuring the zone of inhibition, and resistance was explained in accordance with the EUCAST breakpoints.

Statistical analysis was performed using Microsoft Excel 2016 (Microsoft, USA) and IBM SPSS Statistics 26.0 (IMB, USA). Results were exhibited as interquartile ranges (IQR) as well as median values. The comparison of quantitative data, which do not follow standard distribution between groups, was calculated using the Mann–Whitney U-Test, while Pearson Chi-square and Fisher exact tests were applied on nominal variables to determine associations among them. A p-value of <0.05 was scrutinized as statistically significant. The data was entered in SPSS and validated by an additional statistical analyst for reliability.

3. Results

Depending on bacteriological as well as intraoperative findings, there was an establishment of two patient groups (Table 1). The AcA group consisted of 49 patients (52.7%) with a positive culture sample from the peritoneal cavity whereas those with a negative culture were classified in the AsA group, this group consisting of 44 patients (47.3%). *E. coli* was identified in 79 patients (84.9%), thus it is the most common representative of appendiceal intraluminal microbiota in simple and complex appendicitis. *P. aeruginosa* was the most prevalent microorganism of the extraluminal appendiceal microbiota (AcA/AsA: 15/5).

Table 1. Overview of study population.

	AcA	AsA	Total	*p*-Value
Children, *n* (%)	49 (52.7)	44 (47.3)	93	0.269
Age, median (IQR)	12 (9–14)	13 (10–15)	-	0.194
Laboratory values, median (IQR)				
WBC count ($\times 10^9$/L),	17.01 (13.75–20.25)	14.79 (13.20–16.76)	-	0.019
CRP (g/L),	25.93 (4.50–89.68)	15.82 (2.86–39.29)	-	0.201
Neu	84.50 (80.93–87.00)	80.80 (73.90–84.80)		0.012
Alvarado Score, points, median (IQR)	8 (7–9)	7 (6–9)	-	0.098
Type of surgery, *n* (%)				
Laparotomy	7 (63.6)	4 (36.4)	11	
Laparoscopy	42 (51.2)	40 (48.8)	82	0.439
Drainage tube, *n* (%)	30 (76.9)	9 (23.1)	39	<0.001
Length of hospital stay, days, median (IQR)	6 (4–9)	5 (4–6)	-	0.002

AsA = acute simple appendicitis, AcA = acute complex appendicitis, WBC = White Blood Cells, CRP = C-Reactive Protein. Median values are presented with IQR (25%, 75%).

A majority of the patients (76.9%), who had an inserted drainage tube, were diagnosed with AcA ($p < 0.001$) (Table 1). In a comparison between both groups, it was suggested that AuA had a slightly shorter median postoperative hospital stay, of five versus six days. The clinical characteristics of patients are shown in Table 1.

In Table 2, the number of the prevalent isolates per group is shown, AcA and AsA, respectively. A total of 25 different species were identified from samples obtained from patients with AsA, while 38 species were identified from samples of patients with AcA. The most commonly isolated isolate from the appendices were *E. coli*, which was found in 79 samples, followed by *P. aeruginosa* found in 20 samples, *Sphingomonas paucimobilis* (*S. paucimobilis*) in 9 samples, *Klebsiella pneumoniae* (*K. pneumoniae*) in 7 samples, *Bacterioides fragilis* (*B. fragilis*) in 5 samples and *Citrobacter braakii* (*C. braakii*) in 3 samples (Table 2).

Antibacterial susceptibility testing results are listed in Table 3. A total of 79 isolates of *E. coli* were identified and varied in antibacterial susceptibility. All strains were susceptible to meropenem and amikacin. Five (8.5%) strains were resistant to ceftazidime; thirty-two (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; eighteen (30.5%) to amoxicillin/clavulanic acid; one (1.7%) to piperacillin/tazobactam; and one (1.7%) to gentamicin. Additionally, five ESBL-producing strains of *E. coli* were also isolated.

Table 3. Antimicrobial resistance and susceptibility of isolated pathogens.

	E. coli n, %		*P. aeruginosa* n, %		*Klebsiella* n, %		*Citrobacter* n, %	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**
CAZ	5 8.5	54 91.5	5 26.3	14 73.7	1 11.1	8 88.9		5 100.0
AMP	32 54.2	27 45.8	15 78.9	4 21.1	7 77.8	2 22.2		5 100.0
CTX	6 10.2	53 89.8	12 63.2	7 36.8		9 100.0		5 100.0
MRP		59 100.0		19 100.0	1 11.1	8 88.9		5 100.0
IMI	6 10.2	53 89.8	7 36.8	12 63.2	1 11.1	8 88.9		5 100.0
AK		59 100.0		19 100.0		9 100.0		5 100.0
CN	1 1.7	58 98.3		19 100.0		9 100.0		5 100.0
CIP	8 13.6	51 86.4	2 10.5	17 89.5	1 11.1	8 88.9		5 100.0
C	6 10.2	53 89.8	10 52.6	9 47.4		9 100.0		5 100.0
ETP	2 3.4	57 96.6	12 63.2	7 36.8	1 11.1	8 88.9		5 100.0
AUG	18 30.5	41 69.5	16 84.2	3 15.8	2 22.2	7 77.8	5 100.0	
TZP	1 1.7	58 98.3	2 10.5	17 89.5	1 11.1	8 88.9		5 100.0

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.

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 3. *Citrobacter* spp. tested resistant to all antibiotics with the exception of amoxicillin/clavulanic acid, while *Klebsiella* spp. was resistant to cefotaxime, amikacin, gentamicin as well as chloramphenicol.

4. Discussion

The choice of the correct empirical antibacterial therapy is complex as it requires a clinician to decide on the most suitable antibiotic treatment prior to receiving the results of

In the past ten-year period, antibiotic combinations of cefatizidime/avibactam, ceftolozane/tazobactam and piperacillin/tazobactam have been explored as prospective treatment options [20].

Avibactam is an affiliate of the class of azabicycloalkanes. Avibactam is a non-beta-lactam beta-lactamase inhibitor that is accessible in combination with ceftazidime. This combination was approved by the Food and Drug Administration (FDA) on 25 February 2015 for the treatment of complex intra-abdominal infections in combination with metronidazole [21]. This combination has shown an efficacy of up to 90% against ceftazidime-resistant strains of *P. aeruginosa* [22]. Combined treatment with ceftazidime-avibactam and colistin has shown promise in treating infections with XDR (extremely drug-resistant) *P. aeruginosa* [23]. Levels of antimicrobial resistance to ceftazidime and avibactam across different regions do not show significant variability. They retain sufficient activity against Gram-negative bacteria, especially the *Enterobacteriaceae* family. *P. aeruginosa* is less susceptible to ceftazidime and avibactam compared to *Enterobacteriaceae*. Ceftazidime and avibactam cannot be used against microorganisms with intrinsic resistance. Strains that display resistance to ceftazidime and avibactam should be treated with other effective antimicrobials or in combination with other antibiotics [14].

Ceftolozane/tazobactam was accepted by the FDA in 2014, shortly before ceftazidime/avibactam was approved for the same indications. It is highly effective in combinations with meropenem and levofloxacin [20]. Nevertheless, antimicrobial resistance remains an issue with around 10% of *P. aeruginosa* strains displaying resistance to ceftolozane/tazobactam [24].

The combination of piperacillin/tazobactam includes an anti-pseudomonal penicillin and a beta-lactamase inhibitor. The mechanism of action is based on inhibition of biosynthesis of mucopeptides of the cell wall by binding to one or multiple penicillin-binding proteins. The antibiotic is highly effective during the growth or log stage [25,26]. Treatment protocols have extensive variations, yet most commonly include in-hospital treatment for one to two days (e.g., ceftriaxone/metronidazole, piperacillin/tazobactam or ciprofloxacin/metronidazole) until symptoms are resolved and the WBC count is normalized. This is followed by oral antibiotic therapy in the outpatient setting (e.g., amoxicillin/clavulanic acid or ciprofloxacin and metronidazole) [27].

Amikacin in our study demonstrated significant efficacy against isolates from the samples. It is a broad-spectrum semi-synthetic aminoglycoside antibiotic, derived from kanamycin with antimicrobial properties. Amikacin is bound irreversibly to the bacterial 30S ribosomal subunit, subsequently locking 16S rRNA and S12 protein within the 30S subunit. This leads to interference with the translational initiation complex and misreading of mRNA, thereby hampering protein synthesis and resulting in a bactericidal effect. This agent is usually used for short-term treatment of severe infections due to susceptibility of various strains of Gram-negative bacteria [28]. Data are scarce regarding amikacin-resistant *Pseudomonas* spp. Loho et al. showed that only two *P. aeruginosa* isolates were resistant against amikacin. Its amalgam with doripenem is synergistic and improves treatment results [29].

The most confined microorganism from our patients' samples was *E. coli*, especially in those treated for acute simple appendicitis. This finding concurs with results obtained by other authors [4,8,30–34]. Our facts reveal that strains of *E. coli* are sensitive to antibacterial agents such as amikacin, and meropenem, which are in line with recent studies by other researchers [31]. Strains resistant to other antibacterial agents included in the treatment algorithms were also discovered in this study, such as cefotaxime and ceftazidime. In total, 6 strains of 49 were found to be resistant to cefotaxime and 5 strains to ceftazidime. Only five isolates (8.5%) were ESBL-positive. This falls in with the data from other studies determining the prevalence of ESBL-producing *E. coli* in Latvia. Data from the leading hospitals in Latvia showed a decline in the number of ESBL-producing *Enterobacteriaceae* in 2020 when compared with data collected in 2017. About 15–20% of *E. coli* isolates displayed ESBL activity [32].

To prevent the continued spread of infection in cases of acute appendicitis with complications, such as perforation, empirical treatment could include ceftriaxone (in combination with metronidazole) or ertapenem for children above the age of 1 month. Other options for empirical treatment could include piperacillin/tazobactam, imipenem or meropenem. The main aim of an appropriate antibacterial treatment regimen is to prevent complications associated with infection. Empirical antibiotic treatment should be based on information about the most commonly isolated microorganisms in a specified region and their profile of antibacterial resistance [34].

The purpose of using ceftazidime for non-surgical treatment of simple appendicitis is to limit bacterial growth associated with *P. aeruginosa* within the appendix, in order to prevent the destruction of the appendiceal wall and its subsequent perforation. Further research is necessary to determine what practical implications the findings of our study have.

The study had some limitations and deserves a word. Although the study was conducted in one of the biggest tertiary children's hospitals in the country, we faced a limited number of resources in the odd hours, courier services, microbiological laboratory working hours, the COVID-19 pandemic and, lastly, in the beginning, the ethics committees' non-approval for children below the age of seven years.

5. Conclusions

E. coli is the main causative agent of acute appendicitis in children demonstrating susceptibility to various antibiotics. *P. aeruginosa* is identified more frequently in cases of acute complex appendicitis compared to cases of acute simple appendicitis. *P. aeruginosa* is susceptible to agents of the cephalosporin group, such as ceftazidime, however, *P. aeruginosa* has phenotypic resistance to cefotaxime, which was also confirmed in our study. Therefore, cefotaxime should be removed from the guidelines for empirical treatment of acute appendicitis. Antibiotics with distinct implementation should be recommended for the treatment of acute complex appendicitis to prevent the development of antimicrobial resistance.

Author Contributions: Study conception and design were developed by M.K., A.E., A.P., J.K., A.R. and A.S. Data acquisition was carried out by M.K., M.V., S.O.S. and R.B. Analyses and data interpretation were performed by M.K., A.R., R.B., L.A. and J.K. This article's manuscript was drafted by A.R., M.K. and J.K. Critical revision was executed by all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by Latvian Academy of Science and Riga Stradins University.

Institutional Review Board Statement: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Riga Stradins University, reference number: 21/27.04.2017. and Children's Clinical University Hospital, reference number: SP- 37/2018.), and with the 1975 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Statement: Written informed consent was obtained from all subjects and their parents involved in the study.

Acknowledgments: The authors would like to acknowledge every single person, including the Latvian Council of Science and Riga Stradins University, for their sincere contribution in funding this project.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Aiyoshi, T.; Masumoto, K.; Tanaka, N.; Sasaki, T.; Chiba, F.; Ono, K.; Jimbo, T.; Urita, Y.; Shinkai, T.; Takayasu, H.; et al. Optimal First-Line Antibiotic Treatment for Pediatric Complicated Appendicitis Based on Peritoneal Fluid Culture. *Pediatr. Gastroenterol. Hepatol. Nutr.* **2021**, *24*, 510–517. [CrossRef] [PubMed]
2. Bhattacharya, J.; Silver, E.J.; Blumfield, E.; Jan, D.M.; Herold, B.C.; Goldman, D.L. Clinical, Laboratory and Radiographic Features Associated With Prolonged Hospitalization in Children With Complicated Appendicitis. *Front. Pediatr.* **2022**, *10*, 828748. [CrossRef] [PubMed]

3. Schulin, S.; Schlichting, N.; Blod, C.; Opitz, S.; Suttkus, A.; Stingu, C.S.; Barry, K.; Lacher, M.; Bühligen, U.; Mayer, S. The intra- and extraluminal appendiceal microbiome in pediatric patients. *Medicine* **2017**, *96*, e9518. [CrossRef]
4. European Committee on Antimicrobial Susceptibility Testing. Clinical Breakpoints and Dosing of Antibiotics. Available online: http://www.eucast.org/clinical_breakpoints/ (accessed on 10 December 2020).
5. Abdussemee, A.; Oludolapo, A.; Adeyinka, A.; Olusegun, F. Bacterial Pattern in Acute Appendicitis. *Ann. Afr. Surg.* **2018**, *15*, 8–13.
6. Parthiban, N.; Harish, M. A study on microbiology culture of acute appendicectomy specimen and its correlation with wound infection. *Int. Surg. J.* **2017**, *4*, 2212–2215. [CrossRef]
7. Song, D.W.; Park, B.K.; Suh, S.W.; Seung, E.L.; Jong, W.K.; Joong-Min, P.; Hye, R.K.; Mi-Kyung, L.; Yoo, S.C.; Beom, G.K.; et al. Bacterial culture and antibiotic susceptibility in patients with acute appendicitis. *Int. J. Colorectal. Dis.* **2018**, *33*, 441–447. [CrossRef] [PubMed]
8. Rekomendācijas Antibakteriālo Līdzekļu Lietošanai Ķirurģiskajā Praksē. KS/MET-011-00. Bērnu Klīniskā Universitātes Slimnīca. 2019. Available online: https://www.spkc.gov.lv/lv/media/4147/download (accessed on 29 June 2022).
9. Drugbank Online. Ceftazidime. Available online: https://go.drugbank.com/drugs/DB00438 (accessed on 6 June 2022).
10. Adámková, V.; Marekovič, I.; Szabó, J.; Pojnar, L.; Billová, S.; Horvat Herceg, S.; Kuraieva, A.; Możejko-Pastewka, B. Antimicrobial activity of ceftazidime-avibactam and comparators against *Pseudomonas aeruginosa* and *Enterobacterales* collected in Croatia, Czech Republic, Hungary, Poland, Latvia and Lithuania: ATLAS Surveillance Program, 2019. *Eur. J. Clin. Microbiol. Infect. Dis.* **2022**, *41*, 989–996. [CrossRef] [PubMed]
11. Wang, Y.; Wang, J.; Wang, R.; Cai, Y. Resistance to ceftazidime-avibactam and underlying mechanisms. *J. Glob. Antimicrob. Resist.* **2020**, *22*, 18–27. [CrossRef]
12. University of California San Francisco. Pediatric Appendicitis Clinical Algorithm. Available online: https://idmp.ucsf.edu/content/pediatric-appendicitis-clinical-algorithm (accessed on 7 July 2021).
13. Pilmis, B.; Jiang, O.; Mizrahi, A.; Nguyen Van, J.; Lourtet-Hascoët, J.; Voisin, O.; Le Lorc'h, E.; Hubert, S.; Ménage, E.; Azria, P.; et al. No significant difference between ceftriaxone and cefotaxime in the emergence of antibiotic resistance in the gut microbiota of hospitalized patients: A pilot study. *Int. J. Infect. Dis.* **2021**, *104*, 617–623. [CrossRef]
14. Drusano, G.L.; Neely, M.N.; Yamada, W.M.; Duncanson, B.; Brown, D.; Maynard, M.; Vicchiarelli, M.; Louie, A. The combination of fosfomycin plus meropenem is synergistic for *Pseudomonas aeruginosa* PAO1 in a hollow-fiber infection model. *Antimicrob. Agents Chemother.* **2018**, *62*, e01682-18. [CrossRef]
15. Feng, Y.; Bakker, R.T.; van Hest, R.M.; Hodiamont, C.J.; Brul, S.; Schultsz, C.; ter Kuile, B.H. Optimization of therapy against *Pseudomonas aeruginosa* with ceftazidime and meropenem using chemostats as model for infections. *FEMS Microbiol. Lett.* **2017**, *364*, fnx142. [CrossRef] [PubMed]
16. Gracia, J.; Gruenberg, K.; Nguyen, L.; MacDougall, C. Multidrug-Resistant *Pseudomonas aeruginosa* Infections: Hard to Treat, But Hope on the Horizon? *Contag. Live—Infect. Dis. Today* **2018**, *3*, 9–11. Available online: https://cdn.sanity.io/files/0vv8moc6/contagion/425a39bc4de8063101549f601e679fca9486728a.pdf/Contagion_0218_3.pdf (accessed on 29 June 2022).
17. National Center for Biotechnology Information. PubChem Database. Avibactam, CID=9835049. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Avibactam (accessed on 10 December 2019).
18. Qin, X.; Tran, B.G.; Kim, M.J.; Wang, L.; Nguyen, D.A.; Chen, Q.; Song, J.; Laud, P.J.; Stone, G.G.; Chow, J.W. A randomised, double-blind, phase 3 study comparing the efficacy and safety of ceftazidime/avibactam plus metronidazole versus meropenem for complicated intra-abdominal infections in hospitalised adults in Asia. *Int. J. Antimicrob. Agents* **2017**, *49*, 579–588. [CrossRef]
19. Xipell, M.; Bodro, M.; Marco, F.; Losno, R.A.; Cardozo, C.; Soriano, A. Clinical experience with ceftazidime/avibactam in patients with severe infections, including meningitis and lung abscesses, caused by extensively drug-resistant Pseudomonas aeruginosa. *Int. J. Antimicrob. Agents* **2017**, *49*, 266–268. [CrossRef]
20. Fournier, D.; Carrière, R.; Bour, M.; Grisot, E.; Triponney, P.; Muller, C.; Lemoine, J.; Jeannot, K.; Plésiat, P.; the GERPA Study Group. Mechanisms of resistance to ceftolozane/tazobactam in Pseudomonas aeruginosa: Resultsof the GERPA multicenter study. *Antimicrob. Agents Chemother.* **2021**, *65*, e01117-20. [CrossRef] [PubMed]
21. Medscape. Piperacillin/Tazobactam (Rx). Available online: https://reference.medscape.com/drug/zosyn-piperacillin-tazobactam-342485#10 (accessed on 7 July 2021).
22. Kwok, C.P.D.; Tsui, S.Y.B.; Chan, K.W.E. Updates on bacterial resistance and empirical antibiotics treatment of complicated acute appendicitis in children. *J. Pediatr. Surg.* **2021**, *56*, 1145–1149. [CrossRef] [PubMed]
23. Georgiou, R.; Eaton, S.; Stanton, M.P.; Pierro, A.; Hall, N.J. Efficacy and Safety of Nonoperative Treatment for Acute Appendicitis: A Meta-analysis. *Pediatrics* **2017**, *139*, e20163003. [CrossRef]
24. National Center for Biotechnology Information. PubChem Database. Amikacin, CID=37768. Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Amikacin (accessed on 12 December 2019).
25. Loho, T.; Sukartini, N.; Astrawinata, D.A.W.; Immanuel, S.; Aulia, D.; Priatni, I. In Vitro Antibacterial Interaction of Doripenem and Amikacin against Multidrug-Resistant Acinetobacter Baumannii, Pseudomonas Aeruginosa, and Klebsiella Pneumoniae Isolates. *Can. J. Infect. Dis. Med. Microbiol.* **2018**, *2018*, 1047670. [CrossRef]
26. Essenmacher, A.C.; Nash, E.; Walker, S.K.; Pitcher, G.J.; Buresh, C.T.; Sato, T.S. Stump Appendicitis. *Clin. Pract. Cases Emerg. Med.* **2018**, *2*, 211–214. [CrossRef]
27. Snyder, M.J.; Guthrie, M.; Cagle, S. Acute Appendicitis: Efficient Diagnosis and Management. *Am. Fam. Physician* **2018**, *98*, 25–33.

28. Bazzaz, A.; Lor, D.; Mahdi, N. Impact of Some Antibiotics on Bacteria Isolated from Appendices in Kirkuk Province, Iraq. *Adv. Biosci. Biotechnol.* **2018**, *9*, 1–10. [CrossRef]
29. Rickard, J. Antibiotic Use and Antimicrobial Resistance of Surgical Patients with Peritonitis at a Tertiary Referral Hospital in Rwanda. *Surg. Infect.* **2018**, *19*, 382–387. [CrossRef] [PubMed]
30. Turel, O.; Mirapoglu, S.Y.; Yuksel, M.; Ceylan, A.; Gultepe, B.S. Perforated appendicitis in children: Antimicrobial susceptibility and antimicrobial stewardship. *J. Glob. Antimicrob. Resist.* **2018**, *16*, 159–161. [CrossRef]
31. Mazuski, J.E.; Tessier, J.M.; May, A.K.; Sawyer, R.G.; Nadler, E.P.; Rosengart, M.R.; Chang, P.K.; O'Neill, P.J.; Mollen, K.P.; Huston, J.M.; et al. The Surgical Infection Society Revised Guidelines on the Management of Intra-Abdominal Infection. *Surg. Infect.* **2017**, *18*, 1–76. [CrossRef] [PubMed]
32. Jain, N.; Jansone, I.; Obidenova, T.; Simanis, R.; Meisters, J.; Straupmane, D.; Reinis, A. Antimicrobial Resistance in Nosocomial Isolates of Gram-Negative Bacteria: Public Health Implications in the Latvian Context. *Antibiotics* **2021**, *10*, 791. [CrossRef]
33. European Committee on Antimicrobial Susceptibility Testing. Expected Resistant and Susceptible Phenotypes. Available online: https://www.eucast.org/expert_rules_and_expected_phenotypes/expected_phenotypes/ (accessed on 23 May 2022).
34. Terentjeva, M.; Streikiša, M.; Avsejenko, J.; Trofimova, J.; Kovaļenko, K.; Elferts, D.; Bērziņš, A. Prevalence and Antimicrobial Resistance of *Escherichia coli*, *Enterococcus* spp. and the Major Foodborne Pathogens in Calves in Latvia. *Foodborne Pathog. Dis.* **2019**, *16*, 35–41. [CrossRef]

Fourth Publication

sciendo

PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B,
Vol. 74 (2020), No. 2 (725), pp. 100–105.

DOI: 10.2478/prolas-2020-0016

CULTURE BASED EVALUATION OF MICROBIOTA IN CHILDREN WITH ACUTE APPENDICITIS

Juta Kroiča[1], Aigars Reinis[1], Mohit Kakar[2,4,#], Mathilde Delorme[5], Renārs Broks[1], Lāsma Asare[6], Marisa Berezovska[2,4], Vladislavs Jansins[1], Astra Zviedre[2,4], Arnis Enģelis[2,4], Amulya Saxena[3], and Aigars Pētersons [2,4]

[1] Department of Biology and Microbiology, 16 Dzirciema Str., Rīga Stradiņš University, Rīga, LV-1007, LATVIA

[2] Department of Pediatric Surgery, Children's Clinical University Hospital, 45 Vienības Av., LV-1004, Rīga, LATVIA

[3] Department of Pediatric Surgery, Chelsea Children's Hospital, Chelsea and Westminster NHS Fdn Trust, Imperial College London, London, UK

[4] Department of Pediatric Surgery, Rīga Stradiņš University, 45 Vienības Av., Rīga, LV-1004, LATVIA

[5] Faculty of Medicine, Rīga Stradiņš University, 16 Dzirciema Str., Rīga, LV-1007, LATVIA

[6] Department of Statistics, Rīga Stradiņš University, 23 Kapseļu Str., Rīga, LV-1046, LATVIA

[#] Corresponding author, mohitez@gmail.com

Contributed by Aigars Pētersons

Treatment strategies for acute uncomplicated appendicitis have evolved and now conservative antibacterial treatment is recommended over surgical treatment, especially for paediatric patients. The aim of this study was to evaluate microbiota in paediatric patients with acute uncomplicated and complicated appendicitis, and antibacterial susceptibility of the causative microorganisms. Bacteriological identification was conducted using the VITEK2 analyser. Antibacterial susceptibility tests were performed and the results were evaluated in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) "Clinical breakpoints and dosing of antibiotics" (Version 7.0, January 2019). Serodiagnosis of Yersinia enterocolitica *was performed using indirect haemagglutination. The results revealed differences in microbiota in cases of acute complicated and acute uncomplicated appendicitis.* Pseudomonas aeruginosa *was identified more frequently in cases of acute complicated appendicitis. Mixed culture was prevalent in cases of both acute complicated and acute uncomplicated appendicitis. Very few positive extended spectrum beta-lactamase (ESBL)* Escherichia coli *cultures were identified. Most of strains of* Pseudomonas aeruginosa *were resistant to amoxicillin with clavulanic acid, ertapenem, ampicillin and cefotaxime. Some of* E. coli *isolates were resistant to ampicillin and to amoxicillin with clavulanic acid.*

Key words: *appendicitis, microbiota,* Pseudomonas aeruginosa, Escherichia coli, *extended spectrum beta-lactamase, antibacterial susceptibility.*

INTRODUCTION

Acute appendicitis (AA) is one of the most common paediatric abdominal pathologies. It requires surgery, and despite advances in diagnostics and treatment, a uniform understanding of the aetiology and pathogenesis of appendicitis is still missing (Naher *et al.*, 2013; Essenmacher *et al.*, 2018; Snyder *et al.*, 2018). The physiology of the appendix has also not been fully investigated. It is recognised that the vermiform appendix plays a role in the development and maturation of the immune system (Gebbers and Laissue, 2004; Rhee *et al.*, 2005). Involvement of microbes in the pathogenesis of appendicitis is not entirely understood; however, recent research suggests that the appendix functions as a microbiota reservoir in the gastrointestinal tract. The belief is that it ensures the repopulation of microbiota

during acute illnesses, in which the gastrointestinal tract is colonised by pathogens such acute gastroenteritis, and after antibacterial treatment (Guinane *et al.*, 2013).

Historically, the only effective treatment method of appendicitis and prevention of septic complications has been surgery, namely, an appendectomy, which has been practiced for over 130 years (Rogers *et al.*, 2016). This view has been challenged in recent years as conservative treatment using antibiotics has supplanted surgical interventions (Lamps, 2010). Clinical research has demonstrated the efficacy of antibacterial treatment, but nonetheless, 27% of patients each year still require surgery (Roberts, 1988).

One of the most important issues in paediatric surgery is distinguishing between acute uncomplicated appendicitis (AnA) and acute complicated appendicitis (AcA) upon commencing conservative treatment, as complicated cases indicate a delay in establishing diagnosis and require emergency surgery. These cases constitute more than 35% of all AA cases in Latvia. Our current research showed that the total number of AA cases treated surgically at the Children's Clinical University Hospital has not changed, but there has been an increased incidence of AcA (Kakars *et al.*, 2017). Increase use of conservative treatment necessitates the evaluation of algorithms for antibacterial treatment, as they can differ among clinical institutions (Salo *et al.*, 2017).

The aim of this study was to evaluate microbiota in paediatric patients with acute uncomplicated and complicated appendicitis, and antibacterial susceptibility of the causative microorganisms.

MATERIALS AND METHODS

This study included children between the ages of seven and 17 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. Preoperative screening involved physical examination, complete blood count, abdominal ultrasound (US) and detection of serum values of C-reactive protein (CRP) and interleukin-6 (IL-6).

The paediatric surgery team supervising patients with appendicitis received a written consent form from the caregiver and assent from the patient if they were 13 years of age or older. The consent form provided information to the patient and the caregiver about the research objective and methodology used for examining the biological material.

Ethical approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Rīga Stradiņš University; reference number: 21/27.04.2017; and Children's Clinical University Hospital; reference number: SP-37/2018), and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from a parent of each individual participant included in the study. Clinical data collected prior to surgery included patients' age, sex, and current medical history.

Altogether, a total of 67 patients were eligible and recruited for this study. All of these patients had an appendectomy, and either laparoscopic or conventional laparotomy was performed in all of the patients. Intraoperatively, a microbiological culture swab from the peritoneal cavity was collected. After the removal of the appendix, an extra submucosal swab was taken to avoid bacterial dissemination from the following swabs. The appendix was dissected longitudinally and under sterile conditions, and swab samples were taken from the distal and proximal part of the appendiceal lumen. Each pair was placed in Amies medium for immediate transfer and subsequent bacterial culture (Schulin *et al.*, 2017). They were cultivated under aerobic and anaerobic conditions. Cultivation was performed on blood agar (Supplement, Oxid, UK; defibrinated sheep blood – E&O laboratories limited, Scotland), MacConkey (Oxid, UK) and tripticase soy (Oxid, UK) agar. Bacterial identification was performed using the VITEK2 analyser (Biomerieux, France).

Antibacterial susceptibility tests were performed and the results were evaluated in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), "Clinical breakpoints and dosing of antibiotics" (Version 7.0, January 2019) (Anonymous, 2019). Overnight cultures were suspended in physiological saline to 0.5 McFarland units (McFarland Densitometer DEN-1, Biosan, Latvia). The suspension was inoculated on Mueller-Hinton agar (Oxid, UK). Selected antibiotics were placed on the inoculated plates and included ceftazidime 10 µg, ampicillin 10 µg, cefotaxime 5 µg, meropenem 10 µg, imipenem 10 µg, amikacin 30 µg, gentamicin 10 µg, ciprofloxacin 5 µg, chloramphenicol 30 µg, ertapenem 10 µg, amoxicillin+clavulanic acid 30 µg, and piperacillin+tazobactam 36 µg (Liofilchem, Italy). The plates were incubated at +35 ± 1°C temperature for 18 ± 2 h. A double disk synergy test (DDST) was used to confirm extended spectrum beta-lactamase (ESBL). Disks containing cephalosporins (cefotaxime, ceftazidime) were applied to plates next to a disk with clavulanic acid (amoxicillin-clavulanic acid). A positive result was indicated if the inhibition zones around any of the cephalosporin disks were augmented in the direction of the disk containing clavulanic acid. Results were evaluated by measuring the zone of inhibition, and resistance was interpreted in accordance with the EUCAST breakpoints.

Haematoxylin-eosin staining was performed and the grade of inflammation was assessed, differentiating gangrenous and phlegmonous appendicitis. Serodiagnosis of *Yersinia enterocolitica* was performed using indirect (passive) haemagglutination.

For statistical analysis, Microsoft Excel 2016 and IBM SPSS Statistics 22 were employed. Results were expressed as median values and interquartile ranges (IQR). The com-

parison between groups was made using both the Mann–Whitney U-Test (two groups) and Kruskal–Wallis test (three groups) for non-parametric distribution, and the Fisher Exact Test was applied on normally distributed variables to determine correlations between them. A p-value of < 0.05 was considered statistically significant. The data was entered in SPSS and validated by an additional statistical analyst for accuracy.

RESULTS

A total of 67 children having arrived at the Emergency Department were included in this study (33 males, 34 females). Patient age ranged 7–17 years with a median age of 12 years. Depending on the intraoperative and bacteriological findings, two patient groups were established (Table 1). Patients with positive culture from samples of the peritoneal cavity were classified in the AcA group, which consisted of 34 patients (50.7%). Those patients with a negative culture were classified in the AnA group, which consisted of 33 patients (49.3%). There was no statistically significant difference between the results of the samples taken from the anatomical parts of the appendiceal lumen. *E. coli* was the prevalent representative of appendiceal intraluminal microbiota in both complicated and uncomplicated cases, totalling 51 patients (76.0%). *P. aeruginosa* was the prevalent microorganism of the extraluminal appendiceal microbiota (AcA/AuA:13/1). There were some differences in microbiota of the proximal and distal parts of the appendix between patients with acute complicated and acute uncomplicated appendicitis. In 22 of 34 AcA cases (55.0%) microbiota were identical, but the microbiota in distal and proximal parts differed for the remaining 12 cases (35.0%).

Bacteria were grown from samples of submucosa in both acute complicated and uncomplicated appendicitis. *E. coli* was the prevalent species, with *P. aeruginosa* being the second most commonly isolated microorganism (Table 1). Histological examination of surgically removed specimens showed a significant correlation between gangrenous appendicitis in AcA and phlegmonous appendicitis in AnA (Chi-square = 15.246, df = 1, $p < 0.001$). More than a half of the patients (61.3%) who had a drainage tube inserted were diagnosed with AcA ($p = 0.004$). A simple comparison suggested that AnA had a slightly shorter median postoperative hospital stay of five versus six days. *Yersinia enterocolitica* antibody detection preoperatively was negative in all cases. The remaining demographics and clinical characteristics of the patients are shown in Table 2.

Bacterial culture resulted in positive intraluminal samples with growth of one or several strains from each appendix. Table 2 shows the number of cases of the most common isolates for the AcA and AnA patient groups. Frequently, mixed strains were found at culture. The most common bacteria isolated from the appendix were *Escherichia coli* in 46 (37.4%), followed by *Pseudomonas aeruginosa* in 16 (13.0%), *Klebsiella pneumoniae* in nine (7.3%), *Bacterioides fragilis* in six (4.9%), and *Citrobacter braakii* in four

Table 1. Types of isolated bacteria, frequency and percentage in acute complicated appendicitis and acute uncomplicated appendicitis

	AcA		AnA		Total isolates No.	p-value
	No.	%	No.	%		
Escherichia coli	24	47.1	27	52.9	51	0.460#
Pseudomonas aeruginosa	13	92.9	1	7.1	14	0.098*
Klebsiella pneumoniae	3	50.0	3	50.0	6	0.480
Citrobacter braakii	0	0	3	100.0	4	0.053*
Bacterioides fragilis	1	20.0	4	80.0	5	0.321
Mixed cases	23	51.1	22	48.9	45	0.867
Total	60		63		123	

AcA, acute complicated appendicitis; AnA, acute uncomplicated appendicitis, # Pearson Chi-square test, * Fisher Exact test

Table 2. Characteristics and distribution of the study population

	AcA	AnA	Total	p-value
Children, n (%)	34 (50.7)	33 (49.3)	67	0.288
Age, median (IQR)	12 (9–15)	11 (10–15)	–	0.200
Laboratory values				
WBC count ($\times 10^9$/l),	16.7	14.5	–	< 0.001
CRP (g/l),	27.6	10.3	–	0.050
Duration of symptoms, h (IQR)	28 (16–51)	20 (12–26)	–	0.038
Alvarado score, points	8	7	–	< 0.001
Type of surgery, n (%)				
Laparotomy	7 (63.6)	4 (36.4)	11	0.349
Laparoscopy	26 (48.1)	28 (51.9)	54	
Histology, n (%)				
Phlegmonous	10 (29.4)	24 (70.6)	34	< 0.001
Gangrenous	23 (74.2)	8 (25.8)	31	
Drainage tube, n (%)	19 (70.4)	8 (29.6)	27	0.008
Length of hospital stay, days (IQR)	6 (5–9)	5 (4–6)	–	0.006

AcA, acute complicated appendicitis; AnA, acute uncomplicated appendicitis; IQR, interquartile range; WBC, white blood cells; CRP, C-reactive protein. Median values are presented with IQR (25%, 75%)

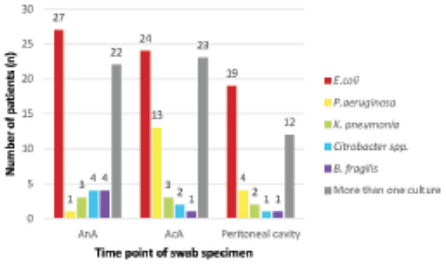


Fig 1. Types of organisms isolated.

samples (3.3%) (Table 2, Fig. 1). The 46 samples isolating *E. coli* had various antibacterial sensitivities: four (~8.7%) strains were resistant to ceftazidime; 17 (~37%) to ampicillin; three (~6.5%) to cefotaxime; one (~2.17%) to imipenem; four (~8.7%) to ciprofloxacin; three (~6.5%) to chloramphenicol; two (~4.34%) to ertapenem; nine (~19.5%) to

amoxicillin/clavulanic acid, and two (~4.34%) to piperacillin-tazobactam. All strains were susceptible to meropenem, amikacin and gentamicin. Additionally, two ESBL-producing strains of *E. coli* were also isolated.

P. aeruginosa was isolated from 15 samples. Antibacterial resistance was shown for four (26.7%) strains to ceftazidime; 11 (73.3%) to ampicillin; eight (53.3%) to cefotaxime; five (33.3%) to imipenem; five (33.3%) to chloramphenicol; 11 (73.3%) to ertapenem; 11 (73.3%) to amoxicillin/clavulanic acid and one (6.67%) to piperacillin-tazobactam. All strains were susceptible to meropenem, amikacin, gentamicin and ciprofloxacin. Other bacteria from the *Enterobacteriaceae* family were also isolated, such as *Citrobacter spp*., *Klebsiella spp*., and others. *Citrobacter spp.* were resistant to amoxicillin/clavulanic acid, and *Klebsiella spp.* were resistant to ampicillin.

DISCUSSION

Research has shown that appendiceal luminal obstruction, by creating a closed loop, is the cause of appendicitis. Lymphoid hyperplasia in the follicles of the submucosa is the typical cause of luminal obstruction in children. Faecolith is mentioned as a precipitating obstructive factor (Bhangu *et al.*, 2015), while parasites (e.g. nematodes) and inflammatory constrictions are less common causes. Obstruction leads to increased bacterial proliferation, thus increasing the intraluminal pressure that subsequently impedes blood flow, which results in congestion and ischemia that promotes bacterial colonisation.

Although obstruction is the leading theory behind pathogenesis of appendicitis, it is not fully consistent with the data obtained in research and clinical practice. Therefore, bacteria are believed to also play a role in the pathogenesis. There is also a hypothesis highlighting the importance of genetic predisposition as the prevalence of appendicitis is higher among first-degree relatives. Finally, perforated and non-perforated appendicitis, which represent disease progression from an early to a late stage, are epidemiologically recognised as two distinct processes (Bhangu *et al.*, 2015; Abdussemee *et al.*, 2018; Essenmacher *et al.*, 2018). There is a dispute regarding whether the most common causative agents are *E. coli* and anaerobic *Clostridium perfringens* (Abdussemee *et al.*, 2018), as opposed to *Klebsiella spp.* and *Enterobacter spp*. (Parthiban and Harish 2017), or *Bacteroides fragilis, Pseudomonas aeruginosa* and *Peptostreptococcus spp*. (Roberts, 1988; Lamps, 2010; Guillet-Caruba *et al.*, 2011; Chen *et al.*, 2012).

The results of our study demonstrated that 53.0% of *P. aeruginosa* isolates were resistant to cefotaxime, and about a third against ceftazidime. Cefotaxime is included in the guidelines of the Children's Clinical University Hospital for treatment of intraperitoneal infections in paediatric patients (Anonymous, 2013). Although both antibiotics belong to third generation cephalosporins, ceftazidime has shown more efficacy in the treatment of infections with gram negative bacteria, especially *Pseudomonas spp*., an important causative agent of nosocomial infections (Anonymous, 2019). Treatment typically involves the use of beta-lactam antibiotics; however, ceftazidime is the most important antibacterial agent, which functions as a cell wall synthesis inhibitor by binding to penicillin-binding protein 3 (PBP3) (Koss *et al.*, 2016). Nevertheless, ceftazidime-resistant strains have been discovered. Resistance mechanisms against ceftazidime in *P. aeruginosa* include the production of beta-lactamase encoded by genes acquired via horizontal gene transfer, or by increased production of a drug-induced, broad-spectrum, chromosomally encoded class C beta lactamase with an altered affinity (Fisher and Mobashery, 2014). The frequency of resistance against ceftazidime in isolates can fluctuate between 35% to 86% (Du *et al.*, 2010; Noha *et al.*, 2015).

In our study, *P. aeruginosa* was prevalent in samples obtained from patients with acute complicated appendicitis. All strains were sensitive to meropenem, which inhibits cell wall synthesis and is not affected by beta-lactamase (Baldwin *et al.*, 2008). Drusano *et al.* (2018) investigated the potential use of fosfomycin in the treatment of infections with *P. aeruginosa* and discovered that the bacteria rapidly developed resistance against fosfomycin. Therefore, they suggested switching treatment from monotherapy to combination therapy with fosfomycin and meropenem. A synergistic effect was observed with fosfomycin eradicating the meropenem-resistant mutants and meropenem working against fosfomycin-resistant strains. Thus, this combination was recommended as a treatment strategy for wider use in the future (Drusano *et al.*, 2018). Another combination displaying promising results in research settings is meropenem in conjunction with ceftazidime (Feng *et al.*, 2017). In the past decade, the following antibiotic combinations have been investigated as potential treatment options (Gracia *et al.* 2018).

Ceftazidime-Avibactam. Avibactam is a member of the class of azabicycloalkanes. Avibactam is a non-beta-lactam beta-lactamase inhibitor that is available in combination with ceftazidime (Avycaz). This combination was approved by the Food and Drug Administration (FDA) on 25 February 2015 for the treatment of complicated intra-abdominal infections in combination with metronidazole (Anonymous, 2019). This combination has shown efficacy of up to 90% against ceftazidime-resistant strains of *P. aeruginosa* (Mazuski *et al.*, 2016; Qin *et al.*, 2017). Combined treatment with ceftazidime-avibactam and colistin has shown promise in treating infections with XDR (extremely drug-resistant) *P. aeruginosa* (Xipell *et al*, 2017).

Ceftolozane-Tazobactam. Ceftolozane-tazobactam was approved by the FDA in 2014, shortly before ceftazidime-avibactam was approved for the same indications. It is highly effective in combinations with meropenem and levofloxacin (Solomkin *et al.*, 2015; Wagenlehner *et al.*, 2015; Gracia *et al.*, 2018).

In our study, amikacin demonstrated significant efficacy against isolates from samples. It is a broad-spectrum semi-

synthetic aminoglycoside antibiotic, derived from kanamycin with antimicrobial properties. Amikacin irreversibly binds to the bacterial 30S ribosomal subunit, specifically locking 16S rRNA and S12 protein within the 30S subunit. This leads to interference with the translational initiation complex and misreading of mRNA, thereby hampering protein synthesis and resulting in the bactericidal effect. This agent is usually used in short-term treatment of serious infections due to susceptible strains of Gram-negative bacteria (Anonymous, 2019). Data is scarce regarding amikacin-resistant *pseudomonas*. Research conducted by Loho *et al.* (2018) showed that only two of 20 *P. aeruginosa* isolates were resistant against amikacin. Its combination with doripenem is synergistic and improves treatment results.

The most commonly isolated microorganism from our patients' samples was *E. coli*, especially from those treated for acute uncomplicated appendicitis. This finding concurs with results obtained by other authors (Naher *et al.*, 2013; Bhangu *et al.*, 2015; Abdussemee *et al.*, 2018; Bazzaz *et al.*, 2018; Essenmacher *et al.*, 2018; Rickard *et al.*, 2018; Snyder *et al.*, 2018; Turel *et al.*, 2018). Our data revealed that strains of *E. coli* are sensitive to antibacterial agents such as amikacin, imipenem, and meropenem, which is in line with recent studies by other researchers (Bazzaz *et al.*, 2018). There were strains resistant against other antibacterial agents included in the treatment guidelines; three and four of 47 were resistant to cefotaxime and ceftazidime, respectively. Only two isolates were ESBL-positive. This complies with data from other studies that showed the prevalence of ESBL-producing *E. coli* in Latvia. 11% of *E. coli* present in animal microbiota produce ESBL (Terentjeva *et al.*, 2019), whereas in the adult population, ESBL are produced by only 1.6% of *E. coli* (Ny *et al.*, 2018).

CONCLUSIONS

In cases of acute complicated appendicitis, *P. aeruginosa* is the prevalent microorganism, whereas *E. coli* is the most commonly isolated microorganism in acute uncomplicated appendicitis. Antibiotic treatment strategies in cases of acute complicated appendicitis should include antibiotics with different mechanisms of action to achieve a synergistic effect and prevent the development of antibiotic resistance. Mixed culture results were prevalent in both acute complicated and acute uncomplicated appendicitis. The incidence of ESBL producing microorganisms was low in these acute appendicitis cases.

ACKNOWLEDGEMENTS

The authors would like to acknowledge every single person including the Latvian Council of Science and Riga Stradiņš University for their sincere contribution in funding this project. The study was funded by the Latvian Academy of Sciences and Riga Stradiņš University.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdussemee, A., Oludolapo, A., Adeyinka, A., Olusegun, F. (2018). Bacterial pattern in acute appendicitis. *Ann. Afr. Surg.*, **15** (1), 8–13.

Anonymous (2013). *Recommendations for preoperative antimicrobial prophylaxis. Recommendations for the use of antibacterial agents in surgical prophylaxis KSMET-43-00/2013.* Children's Clinical University Clinic, pp. 9.

Anonymous (2019). Clinical breakpoints and dosing of antibiotics. European Committee on Antimicrobial susceptibility testing. http://www.eucast.org/clinical_breakpoints/ (accessed 10 December 2019).

Anonymous (2019). National Center for Biotechnology Information. PubChem Database. Avibactam, CID=9835049. https://pubchem.ncbi.nlm.nih.gov/compound/Avibactam (accessed 10 December 2019).

Anonymous (2019). National Center for Biotechnology Information. PubChem Database. Amikacin,CID=37768. https://pubchem.ncbi.nlm.nih.gov/compound/Amikacin (accessed 12 December 2019).

Anonymous (2019). The Drugbank. Ceftazidime. https://www.drugbank.ca/drugs/DB00438 (accessed 10December 2019).

Baldwin, C. M., Lyseng-Williamson, K. A., Keam, S. J. (2008). Meropenem: A review of its use in the treatment of serious bacterial infections. *Drugs*, **68** (6), 803–838.

Bazzaz, A., Lor, D., Mahdi, N. (2018). Impact of some antibiotics on bacteria isolated from appendices in Kirkuk Province, Iraq. *Adv. Biosci. Biotechnol.*, **9**, 1–10.

Bhangu, A., Søreide, K., Di Saverio, S., Assarsson, J. H., Drake, F. T. (2015). Acute appendicitis: Modern understanding of pathogenesis, diagnosis, and management. *Lancet*, **386** (10000), 1278–1287.

Chen, C. Y., Chen, Y. C., Pu, H. N., Tsai, C. H., Chen, W. T., Lin, C. H. (2012). Bacteriology of acute appendicitis and its implication for the use of prophylactic antibiotics. *Surg. Infect. (Larchmt)*, **13** (6), 383–390.

Drusano, G. L., Neely, M. N., Yamada, W.M., Duncanson, B., Brown, D., Maynard, M., Vicchiarelli, M., Louie, A. (2018). The combination of fosfomycin plus meropenem is synergistic for *Pseudomonas aeruginosa* PAO1 in a hollow-fiber infection model. *Antimicrob. Agents Chemother.*, **62** (12), e01682-18.

Du, S.-J., Kuo, Cheng, C. H., Fei, A., Wei, H.-W., Chang, S.-K. (2010). Molecular mechanisms of ceftazidime resistance in *Pseudomonas aeruginosa* isolates from canine and human infections. *Original Paper Veterin. Med.* **55**, 172–182.

Essenmacher, A. C., Nash, E., Walker, S. K., Pitcher, G. J., Buresh, C. T., Sato, T. S. (2018). Stump appendicitis. *Clin. Pract. Cases Emerg. Med.*, **2** (3), 211–214.

Feng, Y., Bakker, R. T., van Hest, R. M., Hodiamont, C. J., Brul, S., Schultsz, C., ter Kuile, B. H. (2017). Optimization of therapy against *Pseudomonas aeruginosa* with ceftazidime and meropenem using chemostats as model for infections, *FEMS Microbiol. Lett.*, **364** (14), fnx142.

Fisher, J. F., Mobashery, S. (2014). The sentinel role of peptidoglycan recycling in the β-lactam resistance of the Gram-negative Enterobacteriaceae and Pseudomonas aeruginosa. *Bioorg. Chem.*, **56**, 41–48.

Gebbers, J. O., Laissue, J. A. (2004). Bacterial translocation in the normal human appendix parallels the development of the local immune system. *Ann. NY Acad. Sci.*, **1029**, 337–343.

Gracia, J., Gruenberg, K., Nguyen, L., MacDougall, C. (2018). Multidrug-resistant *Pseudomonas aeruginosa* Infections: Hard to treat, but hope on the horizon? *Contagion Live – Infectious Diseases Today*, received from ContagionLive, Database on the World Wide Web. https://www.contagionlive.com/publications/contagion/2018/february/multidrugresistant-pseudomonas-aeruginosa-infections-hard-to-treat-but-hope-on-the-horizon (accessed 10 December 2019).

Guillet-Caruba, C., Cheikhelard, A., Guillet, M., Bille, E., Descamps, P., Yin, L., Khen-Dunlop, N., Zahar, J. R., Sarnacki, S., Revillon, Y., Berche, P., Ferroni, A. (2011). Bacteriologic epidemiology and empirical treatment

of pediatric complicated appendicitis. *Diagn. Microbiol. Infect. Dis.*, **69** (4), 376–381.

Guinane, C. M., Tadrous, A., Fouhy, F., Ryan, C. A., Dempsey, E. M., Murphy, B., Andrews, E., Cotter, P. D., Stanton, C., Ross, R. P. (2013). Microbial composition of human appendices from patients following appendectomy. *MBio.*, **4** (1), e00366-12.

Jackson, H. T., Mongodin, E. F (2014). Culture-independent evaluation of appendix and rectum microbiomes in children with and without appendicitis. *PLOS One*, **9** (4), 1–9.

Kakars, M., Rekevica, A., Engelis, A., Petersons, A. (2017). An update of a single center review in the management of acute appendicitis in children. In: *International Scientific Conference*, 6–7 April 2017, Riga, Latvia. Riga Stradiņš University, Riga, p. 59.

Kos V. N., McLaughlin R. E., Gardner H. A. (2016). Elucidation of mechanisms of ceftazidime resistance among clinical isolates of *Pseudomonas aeruginosa* by using genomic data. *Antimicrobial agents and chemotherapy*, **60** (6), 3856–3861.

Lamps, L. W. (2010). Infectious causes of appendicitis. *Inf. Dis. Clin. North Amer.*, **24** (4), 995-1018-ix-x.

Loho, T., Sukartini, N., Astriwinata, D. A. W, Immanuel, S., Aulia, D., Priatni, I. (2018). *In vitro* antibacterial interaction of Doripenem and Amikacin against multidrug-resistant *Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* isolates. *Can. J. Infect. Dis. Med. Microbiol.* Article ID 1047670, 6 pages. https://doi.org/10.1155/2018/1047670.

Mazuski, J. E., Gasink, L. B., Armstrong, J., Broadhurst, H., Stone, G. G., Rank, D., Llorens, L., Newell, P., Pachl, J. (2016). Efficacy and safety of ceftazidime-avibactam plus metronidazole versus meropenem in the treatment of complicated intra-abdominal infection: Results from a randomized, controlled, double-blind, phase 3 program. *Clin. Infect. Dis.*, **62** (11), 1380–1389.

Naher, H. S., Ktah, F. K. (2013). Bacterial profile associated with appendicitis. *Int. Res. J. Medical Sci.*, Vol. **1** (2), 1–4.

Noha, A. H., Amany, H. I. M., Sahar, M. A. E., Hazem, A. W. A. R. (2015). High prevalence of multidrug resistant *Pseudomonas aeruginosa* recovered from infected burn wounds in children. *Arch. Clin. Microbiol.*, **4** (4), 1–7.

Ny, S., Kozlov, R., Dumpis, U., Edquist, P., Grindahl-Yli-Hannuksela, K., Kling, A. N., Lis, D. O., Lübbert, C., Pomorska-Wesolowska, M., Palagin, I., Vilde, A., Vuopio, J., Walter, J., Wisell, K. T., Tegmark, K., NoDARS ESBL-carrier working group. (2018). Large variation in ESBL-producing *Escherichia coli* carriers in six European countries including Russia. *Eur. J. Clin. Microbiol. Infect. Dis.*, **37**, 2347–2354.

Parthiban, N., Harish, M. (2017). A study on microbiology culture of acute appendicectomy specimen and its correlation with wound infection. *Int. Surg. J.*, **4**, 2212–2215.

Qin, X., Tran, B. G., Kim, M. J., Wang, L., Nguyen, D. A., Chen, Q., Song J., Laud, P. J., Stone, G. G. Chow, J. W. (2017). A randomised, double-blind, phase 3 study comparing the efficacy and safety of ceftazidime/avibactam plus metronidazole versus meropenem for complicated intra-abdominal infections in hospitalised adults in Asia. *Int. J. Antimicrob. Agents*, **49** (5), 579–588.

Rhee, K. J., Jasper, P. J., Sethupathi, P., Shanmugam, M., Lanning, D., Knight, K. L. (2005). Positive selection of the peripheral B cell repertoire in gut-associated lymphoid tissues. *J. Exp. Med.*, **201** (1), 55–62.

Rickard, J. (2018). Antibiotic use and antimicrobial resistance of surgical patients with peritonitis at a tertiary referral hospital in Rwanda. *Surg. Infect.*, **19** (4), 382–387.

Roberts, J. P. (1988). Quantitative bacterial flora of acute appendicitis. *Arch. Dis. Childhood*, **63** (5), 536.

Rogers, M. B., Brower-Sinning, R., Firek, B., Zhong, D., Morowitz, M. J. (2016). Acute appendicitis in children is associated with a local expansion of fusobacteria. *Clin. Infect. Dis.*, **63** (1), 71–78.

Salö, M., Marungruang, N., Roth, B., Sundberg, T., Stenström, P., Arnbjörnsson, E., Fåk, F., Ohlsson, B. (2017). Evaluation of the microbiome in children's appendicitis. *Int. J. Colorectal Dis.*, **32** (1), 19–28.

Schulin, S., Schlichting, N., Blod, C., Opitz, S., Sutticus, A., Stingu, C. S., Barry, K., Lacher, M., Bühligen, U., Mayer, S. (2017). The intra- and extraluminal appendiceal microbiome in pediatric patients. *Medicine*, **96**, 52(e9518).

Snyder, M. J., Guthrie, M., Cagle, S. (2018). Acute appendicitis: Efficient diagnosis and management. *Amer. Fam. Physician*, **98** (1), 25–33.

Solomkin, J., Hershberger, E., Miller, B., Popejoy, M., Friedland, I., Steenbergen, J., Yoon, M., Collins, S., Yuan, G., Barie, P. S., Eckmann, C. (2015). Ceftolozane/tazobactam plus metronidazole for complicated intra-abdominal infections in an era of multidrug resistance: results from a randomized, double-blind, phase 3 trial (ASPECT-cIAI). *Clin. Infect. Dis.*, **60** (10), 1462–1471.

Terentjeva, M., Streikiša, M., Avsejenko, J., Trofimova, J., Kovaļenko, K., Elferts, D., Bērziņš, A. (2019). Prevalence and antimicrobial resistance of *Escherichia coli, Enterococcus spp.* and the major foodborne pathogens in calves in Latvia. *Foodborne Pathog. Dis.*, **16** (1), 35–41.

Turel, O. Mirapoglu, S. Y., Yuksel, M., Ceylan, A., Gultepe, B. S. (2018). Perforated appendicitis in children: Antimicrobial susceptibility and antimicrobial stewardship. *J. Global Antimicrob. Resist.*, **16**, 159–161.

Wagenlehner, F. M., Umeh, O., Steenbergen, J., Yuan, G., Darouiche, R. O. (2015). Ceftolozane-tazobactam compared with levofloxacin in the treatment of complicated urinary tract infections, including pyelonephritis: A randomised, double-blind, phase 3 trial (ASPECT-cUTI). *The Lancet*, **385** (9981), 1949–1956.

Xipell, M., Bodro, M., Marco, F., Losno, R. A., Cardozo, C., Soriano, A. (2017). Clinical experience with ceftazidime/avibactam in patients with severe infections, including meningitis and lung abscesses, caused by extensively drug-resistant *Pseudomonas aeruginosa*. *Int. J. Antimicrob. Agents*, **49** (2), 266–268.

Zhong, D., Brower-Sinning, R., Firek, B., Morowitz, M. J. (2014). Acute appendicitis in children is associated with an abundance of bacteria from the phylum Fusobacteria. *J. Pediatr. Surg.*, **49** (3), 441–446.

Received 3 January 2020
Accepted in the final form 13 February 2020

MIKROBIOTAS BAKTERIOLOĢISKA IZVĒRTĒŠANA BĒRNIEM AKŪTA APENDICĪTA GADĪJUMĀ

Pēdējo gadu laikā ir mainījusies akūta nekomplicēta apendicīta ārstēšanas taktika, kuras ietvaros rekomendēta antibakteriālā terapija nevis ķirurģiska operācija, īpaši bērniem. Šī pētījuma mērķis bija izvērtēt mikrobiotu pediatriska vecuma pacientiem ar akūtu komplicētu un nekomplicētu apendicītu, kā arī izvērtēt etioloģisko ierosinātāju antibakteriālo jutību. Darbā tika izmantotas bakterioloģiskās metodes, mikroorganismu identifikācija veikta, izmantojot VITEK2 analizatoru. Antibakteriālās jutības testi tika veikti un rezultāti izvērtēti saskaņā ar Eiropas antimikrobiālās jutības testēšanas (EUCAST, Versija Nr. 7.0, 2019. g. janvāris) rekomendācijām. *Yersinia enterocolitica* serodiagnostikā tika izmantota netiešā hemaglutinācijas redakcija. Pētījuma rezultāti liecina, ka ir microbiota atšķirības akūta komplicēta un nekomplicēta apendicīta gadījumā. Akūta komplicēta apendicīta gadījumā prevalēja *Pseudomonas aeruginosa*. Akūtu apendicītu gadījumā pārsvarā izdalītas mikroorganismu asociācijas. No pētījumā iekļautajiem pacientiem tikai atsevišķos gadījumos tika izdalīti ESBL producētāji. Daļa *Pseudomonas aeruginosa* celmu bija rezistenti pret amoksicilīnu ar klavulānskābi, ertapenēmu, ampicilīnu un cefotaksīmu. Daļa izdalīto *E. coli* celmu bija rezistenti pret ampicilīnu un amoksicilīnu ar klavulānskābi.

Latvian patent for the invention "Method of prognosing risk of development of gangrenous appendicitis in children"

LV 15613

(19) LATVIJAS REPUBLIKAS
PATENTU VALDE

(11) **LV 15613 B1**
(51) Starpt.pat.kl. **A61B 5/00**
G01N 33/68

Latvijas patents izgudrojumam
2007g. 15.februāra Latvijas Republikas likums

(12) **Īsziņas**

(21) Pieteikuma numurs:	LVP2021000038
(22) Pieteikuma datums:	21.06.2021
(43) Pieteikuma publikācijas datums:	20.01.2022
(45) Patenta publikācijas datums:	20.05.2022

(71) Īpašnieks(i):
RĪGAS STRADIŅA UNIVERSITĀTE,Dzirciema iela 16,Rīga,LV

(72) Izgudrotājs(i):
Aigars PĒTERSONS (LV)
Aigars REINIS (LV)
Arnis EŅĢELIS (LV)
Juta KROIČA (LV)
Lāsma ASARE (LV)
Mohits KAKARS (LV)
Renārs BROKS (LV)

(74) Pilnvarnieks vai pārstāvis:
Ludmila IVANOVA,Dzirciema iela 16,Rīga,LV

(54) Izgudrojuma nosaukums: GANGRENOZA APENDICĪTA ATTĪSTĪBAS RISKA NOTEIKŠANAS PAŅĒMIENS BĒRNIEM
METHOD OF PROGNOSING RISK OF DEVELOPMENT OF GANGRENOUS APPENDICITIS IN CHILDREN

(57) Kopsavilkums:

Gangrenoza apendicīta attīstības risku bērniem nosaka pirms ķirurģiskas operācijas, izmantojot "sendviča" tipa ELISA metodi nosakot asins serumā citokīnu, proti, IL-6, NGAL un LRG1 daudzumu.

IZGUDROJUMA APRAKSTS

[001] Izgudrojums attiecas uz medicīnu, proti, uz abdominālo ķirurģiju, un to var izmantot, parādoties akūtām sāpēm vēdera dobumā bērniem, lai noteiktu gangrenoza apendicīta attīstības risku.

Zināmais tehnikas līmenis

[002] Akūts iekaisums apendicīta gadījumā ir visizplatītākā ķirurģiskā slimība bērniem vecumā līdz 18 gadiem. Bērniem apendektomija veido no 70 līdz 75% no ārkārtas ķirurģiskajām manipulācijām. Gangrenoza apendicīta riska noteikšana ir nopietna problēma, jo akūts iekaisums apendicīta gadījumā samērā bieži "atdarina" citu akūtu iekaisumu vēdera dobumā, piemēram, akūtu iekaisumu mezenteriāla limfadenīta (mezenterīta) gadījumā bērniem. Pacienta veselības stāvoklis strauji pasliktinās, parādās paātrināta sirds darbība, paātrināta elpošana, temperatūra paaugstinās līdz 38,5 - 39° C. Pacientam ir asas sāpes vēderā, var būt vemšana. Asins analīze parāda leikocītu skaita palielināšanos līdz 30 000. Sāpes vispirms aptver visu vēdera lejasdaļu, pēc tam "pārvietojas" uz apakšējo labo stūri. Diezgan bieži pacientam tiek diagnosticēts akūts apendicīts. Veic operāciju, un tikai operācijas laikā tiek atklāts mezenteriāls limfadenīts (mezenterīts). Ārstēšana notiek, ievadot vēdera dobumā antibiotiku maisījumu [1, 2].

[003] Akūtam gangrenozam apendicītam raksturīgs strutains iekaisums ar apendiksa sieniņas nekrozi, tās perforāciju un sekojošu peritonītu. Akūta gangrenoza apendicīta diagnoze tiek noteikta tikai pēc apendiksa intraoperatīvās vizuālās novērtēšanas. Jebkuras manipulācijas ar gangrenozi izmainītu apendiksu noslēdzas ar tā plīšanu. Pēdējā laika novērojumi liecina, ka akūtu apendicītu ne vienmēr vajag operēt, īpaši tas attiecas uz bērniem. Bet akūta gangrenoza apendicīta gadījumā operācija ir obligāti nepieciešama. Saistībā ar izklāstīto problēmu ir svarīgi noteikt gangrenoza apendicīta attīstības risku bērniem vecumā līdz 18 gadiem.

[004] Zināms akūta apendicīta attīstības riska noteikšanas paņēmiens bērniem, izmantojot Alvarado skalas kritērijus [3]. Alvarado skalā ietilpst seši klīniskie simptomi un divi laboratorijas testi [4, 5].

[005] Zināms akūta iekaisuma attīstības riska noteikšanas paņēmiens apendicīta gadījumā bērniem, pielietojot novokaīna blokādi [6, 7]. Novokaīna blokāde mazina spazmas dažādos vēdera dobuma orgānos, kas atrodas šajā inervētajā zonā. Zināmais paņēmiens var izraisīt klīnisko izpausmju samazināšanos.

[006] Zināms akūta iekaisuma attīstības riska noteikšanas paņēmiens apendicīta gadījumā bērniem, izmantojot vēdera dobuma orgānu ultraskaņas izmeklēšanu [8]. Veic vēdera dobuma

un asins plūsmas kartēšanu. Konstatējot piedēkļa sieniņas kontrastainu zīmējumu, un, vizualizējot piedēkli "vainaga" formā, tiek noteikts iekaisuma attīstības risks akūtā apendicīta gadījumā bērniem.

[007] Zināms akūta iekaisuma attīstības riska noteikšanas paņēmiens apendicīta gadījumā bērniem, izpētot leikocītus [9, 10]. Nosaka intoksikācijas leikocitāro indeksu, C-reaktīvā olbaltuma līmeni, imūnglobulīna G un imūnglobulīna E līmeni, aprēķina punktus, summē tos pēc formulas un, ja punktu summa ir 5 un vairāk, prognozē akūta apendicīta attīstības risku.

[008] Zināms akūta iekaisuma attīstības riska noteikšanas paņēmiens apendicīta gadījumā pacientiem, izmantojot dažādus biomarķierus un matemātisko algoritmu, lai iegūtu testa novērtējumu [11].

[009] Zināms akūta iekaisuma attīstības riska noteikšanas paņēmiens apendicīta gadījumā ar klīnisko simptomātiku, kas imitē labās puses nieru kolikas [12]. Pacientam ar aizdomām par akūtu apendicītu vienlaikus nosaka laktoferīna koncentrāciju izkārnījumos un urīna paraugos. 100 reizes atšķaidīta fekāliju ekstrakta un urīna paraugi vienādos apjomos 100 µl ar automātisku pipeti tiek ievadīti plāksnes vai sloksnes iedobēs laktoferīna imūnfermentālai analīzei. Ja fekālā laktoferīna koncentrācija ir lielāka par 7500 ng/g un urīnā laktoferīna koncentrācija ir no 0 līdz 60 ng/ml, nosaka akūta apendicīta risku, izslēdzot akūto labās puses nieru patoloģijas risku.

[010] Nav zināms gangrenoza apendicīta attīstības riska noteikšanas paņēmiens bērniem.

Izgudrojuma mērķis un būtība

[011] Izgudrojuma mērķis ir izstrādāt gangrenoza apendicīta vai mezenteriāla limfadenīta attīstības riska noteikšanas paņēmienu bērniem.

[012] Mērķi realizē, pateicoties tam, ka akūta iekaisuma vēdera dobumā gadījumā pirms ķirurģiskās operācijas veikšanas, izmantojot kvantitatīvās "sendviču" tipa ELISA principu, asins serumā un urīnā nosaka citokīnu daudzumu, interleikīnu – 6 (*IL-6*), lipokalīnu (NGAL), alfa-2-glikoproteīnu 1 (LRG1) un, ja interleikīna daudzums asins serumā ir 641 pg/ml un vairāk, lipokalīna daudzums asins serumā ir 251 ng/ml un mazāk, bet alfa-2-glikoproteīna 1 daudzums asins serumā ir 81.4 ng/ml un mazāk, tad pastāv gangrenoza apendicīta attīstības risks.

[013] Zināmi citokīnu (limfocītu signālšūnas) pētījumi, pētot stresa stāvoklī esošas šūnas: 1) interleikin-6 (*IL-6*), 2) olbaltumu lipokalīnu, asociētu ar neitrofilu želatināzi (*Neutrophil gelatinase-associated lipocalin* (*NGAL*)), 3) alfa-2-glikoproteīnu 1 (*Leucine-rich alpha-2-glycoprotein 1* (*LRG1*)).

[014] Interleikin-6 (IL-6) ir iekaisuma pozitīvs citokīns, ko ražo dažādas šūnas, stimulē imūnatbildi, to sintezē makrofāgi un T-šūnas [13].

[015] Olbaltumviela NGAL – lipokalīns, asociēts ar neitrofīlu želatināzi. Atkarībā no dažādiem patoloģiskiem apstākļiem NGAL sekretējas un ekspresējas ar dažādām šūnām, kas atrodas stresa stāvoklī – ar imūnām šūnām, hepatocītiem, elpošanas un gremošanas trakta epitēlija šūnām [14].

[016] Alfa-2-glikoproteīns 1 (LRG1) piedalās signāla mijiedarbībā šūnu adhēzijas un dažādu imūnprocesu attīstības gadījumos [15].

[017] ELISA ir jūtīga metode, jo katra fermenta molekula var pārveidot daudzas substrāta molekulas, kas darbojas kā reakcijas pastiprinātājs. Antigēns ir saistīts ar cietu virsmu, primāra antiviela sasaistās ar antigēnu, bet sekundārā enzīmu-savienota poliklinālā antiviela sasaistās ar primāro antivielu. Enzīms pārvērš substrātu par nokrāsotu galīgo produktu, kas veido precipitātu proporcionāli antivielu daudzumam pacienta asins serumā. Monoklonālā antiviela sasaistās uz cietās virsmas un saista antigēnu, ja tas ir pacienta asins serumā, un substrātu, kas pārvērš par nokrāsoto produktu proporcionāli antivielu daudzumam pētāmajā asins serumā. Kvantitatīvas "sendviču" tipa ELISA reakcijas veikšanai izmanto speciālas cilvēka specifiskās antivielas (*kit*) [16].

[018] Paņēmienu realizē sekojoši.

[019] Veic citokīnu izpēti, izmantojot kvantitatīvo "sendviču" tipa ELISA (*enzyme-linked immunosorbent assay*) principu. Izmanto biomarķierus IL-6, NGAL (ražotājs *R&D Systems, Minneapolis, MN, USA*) un LRG1 (ražotājs *Novus Biologicals, USA*).

[019] Izmanto IL-6 kā biomarķieri reakcijā ar 100 µl asins seruma un 100 µl urīna. Asinīm ņēma 2 ml stobriņu. Urīnam ņēma 15 ml stobriņu.

[020] Izmanto NGAL kā biomarķieri reakcijā ar 2 µl asins seruma un 10 µl urīna. Asinīm ņēma 2 ml stobriņu. Urīnam ņēma 15 ml stobriņu.

[021] NGAL biomarķierī urīna paraugus atšķaida 5 reizes, bet asins seruma paraugus 25 reizes, izmantojot kita kalibrācījas atšķaidītāju. Ja bija paredzama augstāka vai zemāka koncentrācija, tad atšķaidījumi attiecīgi mainījās, lai iekļautos biomarķiera noteikšanas robežās.

[022] LRG1 biomarķierī urīna paraugus atšķaida 200 reizes, bet asins seruma paraugus 10000 reizes, izmantojot biomarķiera atšķaidītāju. Ja bija paredzama augstāka vai zemāka koncentrācija, tad atšķaidījumi attiecīgi mainījās, lai iekļautos biomarķiera noteikšanas robežās.

[023] Paraugus centrifugē ar ātrumu 2000 apgr./min. 10 minūtes 4° C temperatūrā. No asinīm paņem serumu un no urīna tā augšējo slāni. Turpmākiem pētījumiem paraugus sasaldē - 80° C temperatūrā, lai, ilglaicīgi uzglabājot, nenotiktu proteīnu degradācija.

[024] IL-6 biomarķierī izmanto kvantitatīvā "sendviču" tipa ELISA principu. Biomarķierī nodrošinātā mikroplatīte ir pārklāta ar cilvēka specifiskajām IL-6 antivielām. Pirmajā reakcijas solī mikroplatīšu bedrītēs pievieno standartus un paraugus, un šķīdumos esošais IL-6 piesaistās

pie imobilizētajām antivielām. Mazgāšanas solī skalo visas pie antivielām nepiesaistījušās vielas. Pēc tam pievieno cilvēku specifisko IL-6 enzīmu-savienotu poliklonālu antivielu. Pēc atkārtotas mazgāšanas, kad tiek aizvākti nepiesaistījušās antivielas - enzīma reaģenti, pievieno substrāta šķīdumu, un proporcionāli IL-6 daudzumam attīstās krāsas intensitāte. Iekrāsojuma attīstīšanās tiek apturēta, pievienojot stop šķīdumu (2N sērskābe), un krāsas intensitāti mēra mikroplašu fotospektometrā.

[025] NGAL biomarķierī izmanto kvantitatīvu "sendviču" tipa ELISA principu. Biomarķierī nodrošinātā mikroplatīte ir pārklāta ar cilvēka specifiskajām NGAL antivielām. Pirmajā reakcijas solī mikroplatīšu bedrītēs pievieno standartus un paraugus. Šķīdumos esošais NGAL piesaistās pie imobilizētajām antivielām. Mazgāšanas solī skalo visas pie antivielām nepiesaistījušās vielas. Pēc tam pievieno cilvēku specifisko NGAL enzīmu-savienotu poliklonālu antivielu. Pēc atkārtotas mazgāšanas, kad tiek aizvākti nepiesaistījušās antivielas - enzīma reaģenti, pievieno substrāta šķīdumu, un proporcionāli NGAL daudzumam attīstās krāsas intensitāte. Iekrāsojuma attīstīšanās tiek apturēta, pievienojot stop šķīdumu (2N sērskābe), un krāsas intensitāti mēra mikroplašu fotospektometrā.

[026] LRG1 biomarķierī tiek izmanots kvantitatīvās "sendviču" tipa ELISA princips. Biomarķierī tiek nodrošināta 96 bedrīšu mikroplatīte, kas pārklāta ar cilvēka specifiskajām poliklonālajām antivielām. Pirmajā solī pievieno standartus un paraugus, un šķīdumos esošais LRG1 piesaistās imobilizētajām antivielām. Mazgāšanas solī skalo visas nepiesaistījušās vielas. Nākamajā solī pievieno cilvēka LRG1 specifiskās biotinilētās poliklonālās antivielas. Mazgāšanas soli atkārto. Pēc tam pievieno streptavidīna peroksidāzes konjugātu, kas atpazīst biotinilētās poliklonālās antivielās un piesaistās tām. Vēlreiz atkārto mazgāšanas soli un pievieno peroksidāzes enzīma substrātu. Proporcionāli LRG1 daudzumam attīstās krāsas intensitāte. Iekrāsojuma attīstīšanās tiek apturēta, pievienojot stop šķīdumu (0,5 N sālsskābe), un krāsas intensitāti mēra mikroplašu fotospektrometrā.

[027] Tiek izmantotas biomarķieros nodrošinātās 96 bedrīšu mikroplatītes, kas visos gadījumos jau ir pārklātas ar attiecīgā biomarķiera cilvēka specifiskajām poliklonālajām antivielām. Biomarķieru attiecīgie standarti ir liofilizētā veidā, un pirms lietošanas ir jāizšķīdina attiecīgā biomarķiera atšķaidītājā (LRG1) vai dejonizētā/destilētā ūdenī (NGAL). Visas pārējās vielas ir šķidrā koncentrētā veidā, kas jāatšķaida ar destilētu/dejonizētu ūdeni vai attiecīgā biomarķiera atšķaidītāju.

[028] Lai izstrādātu jaunu, ticamu un savlaicīgu akūta gangrenoza apendicīta attīstības riska noteikšanas paņēmienu bērniem līdz 18 gadu vecumam, laika posmā no 2018. gada līdz 2020. gadam VSIA Bērnu klīniskajā universitātes slimnīcā (BKUS) tika veikts, uz klīniskās gaitas, laboratorisko un ārstēšanas rezultātu analīzi pamatots, prospektīvs pētījums. Pētījumā tika

iekļauti 59 pacienti ar akūtām sāpēm vēderā un sākotnējo klīnisko diagnozi "aizdomas par akūtu apendicītu". Pētījuma protokolā "aizdomas par akūtu apendicītu" definēta kā diagnoze pacientam, kuram BKUS Neatliekamās palīdzības nodaļā iegūti un izvērtēti venozo asins paraugi, veikta vizuālā diagnostika (vēdera dobuma orgānu un retroperitoneālās telpas ultrasonogrāfija) un papildus nozīmēta bērnu ķirurga konsultācija, lai apstiprinātu vai izslēgtu akūtu vēdera dobuma ķirurģisku slimību. Pētījuma procesā visiem 59 pacientiem tika veikti citokīnu pētījumi, izmantojot kvantitatīvās "sendviču" tipa ELISA principu. Izmantoja interleikīnu – 6 (IL-6), lipokalīnu (NGAL), un alfa-2-glikoproteīnu 1 (LRG1). Pētījuma procesā 30 pacientiem noteica akūtu mezenteriālu limfadenītu. 29 pacientiem apstiprināja akūta gangrenoza apendicīta attīstības iespēju. Jaunais gangrenoza apendicīta attīstības riska noteikšanas paņēmiens tika izstrādāts, pamatojoties uz 29 pacientu fizikālās, laboratoriskās un radioloģiskās izmeklēšanas datiem. Ķirurģiska ārstēšana bija indicēta visiem 29 bērniem, un 10 bērniem akūta gangrenoza apendicīta diagnoze tika apstiprināta operācijas laikā, kā arī precizēta ar aklās zarnas piedēkļa patohistoloģisko izmeklēšanu.

[029] Tika novērotas statistiski ticamas atšķirības starp akūtu gangrenozu apendicītu un akūtu mezenteriālu limfadenītu. Pētījumam nepieciešamie dati tika apstrādāti ar programmu *SPSS for Windows 19.0*. Lai novērtētu citokīnu interleikīnu – 6 (IL-6), lipokalīnu (NGAL), un alfa-2-glikoproteīnu 1 (LRG1) vidējās vērtības, standartnovirzi un atšķirību starp pētāmo un kontroles grupu, rezultātu statistiskajā apstrādē izmantoja ANOVA un Hi kvādrāta (*Chi square*) metodi. Mainīgo lielumu salīdzināšanai starp dažādām pacientu grupām lietoja "t"testu un Kruskala-Vollisa (*Kruskal-Wallis*) un Manna-Vitnija (Mann-Whitney) testu, izmantoja ROC (*receiver operator characteristics*) līkni, noteica laukumu zem līknes AUC (*area under the curve*) un tā 95 % ticamības intervālu CI (*confidence interval*). P vērtība, kas mazāka par 0,05, tika uzskatīta par statistiski nozīmīgu.

[030] Pētījums VSIA BKUS tika veikts saskaņā ar VSIA BKUS Medicīniskās ētikas komitejas atļauju un Helsinku Deklarācijas prasībām.

Izgudrojuma īstenošanas piemēri

[031] Konkrēti piemēri, kas apstiprināja ticama un savlaicīga akūta gangrenoza apendicīta attīstības riska noteikšanas paņēmiena efektivitāti bērniem līdz 18 gadu vecumā ar sāpēm vēderā.

1. piemērs

[032] Paciente B., meitene 7 g.v., tika stacionēta VSIA BKUS ar akūtām sāpēm vēderā 6 stundas. Novērtējot pacienti, pēc jaunā paņēmiena rādītāju kritērijiem, IL-6 daudzums asins serumā 641 pg/ml, NGAL daudzums asins serumā 251 ng/ml un LRG1 daudzuma asins serumā

81 ng/ml, noteica gangrenoza apendicīta attīstības risku pirmajās 1 – 24 stundās. Pacientei veica konvencionālo apendektomiju 7 stundas 40 minūtes no saslimšanas brīža. Noteica diagnozi – gangrenozs apendicīts, kas apstiprinājās morfoloģiski pēc veiktās operācijas. Bērns pēc izveseļošanās tika izrakstīts no stacionāra.

2. piemērs

[033] Pacients I., zēns, 11 g.v., tika stacionēts VSIA BKUS ar akūtām sāpēm vēderā 24 stundas. Novērtējot pacientu, pēc jaunā paņēmiena rādītāju kritērijiem, IL-6 daudzums asins serumā 922 pg/ml, NGAL daudzums asins serumā 56 ng/ml un LRG1 daudzuma asins serumā 41 ng/ml, noteica gangrenoza apendicīta attīstības risku 24 - 48 stundās. Pacientam veica konvencionālo apendektomiju 53 stundas 20 minūtes no saslimšanas brīža. Noteica diagnozi – akūts gangrenozs, perforējs apendicīts, kas apstiprinājās morfoloģiski pēc veiktās operācijas. Bērns pēc izveseļošanās tika izrakstīts no stacionāra.

Rūpnieciskā izmantošana

[034] Laika periodā no 2018. līdz 2020. gadam VSIA BKUS pētījumā tika iekļauti 59 bērni vecumā no 7 līdz 12 gadiem. Pediatru ķirurģijas brigāde, kas uzrauga pacientus ar apendicītu, saņēma rakstiskas piekrišanas veidlapu, kas sniedza informāciju par šo pētījumu.

[035] Visiem pacientiem bija asas sāpes vēderā, dažiem bija vemšana. Temperatūra paaugstinājās līdz 38,5 – 39,0 ° C. Asins analīze rādīja leikocītu skaita pieaugumu līdz 30000 – 35000. Sāpes aptvēra vēdera apakšējo daļu, un dažiem pacientiem tā pārvietojās uz labo apakšējo stūri.

[036] Visiem pacientiem veica izmeklējumus, lai apstiprinātu vai izslēgtu diagnozi: novērtēja fizisko stāvokli, veica pilnu asins analīzi, noteica C-reaktīvo proteīnu asins serumā un veica vēdera dobuma ultraskaņu.

[037] Visiem 59 pacientiem tika veikti citokīnu pētījumi, izmantojot kvantitatīvās "sendviču" tipa ELISA principu. Izmantoja interleikīnu – 6 (IL-6), lipokalīnu (NGAL), un alfa-2-glikoproteīnu 1 (LRG1).

[038] Izmantojot jauno paņēmienu, 10 bērniem (no kopējā skaita) konstatēja akūta gangrenoza apendicīta attīstības riska iespējas, un savlaicīgi 1 – 72 stundu laikā veica apendektomijas operāciju. Ticama, akūta gangrenoza apendicīta attīstības riska iespējas apstiprināja morfoloģiski – pēc operācijas veikšanas. Ņemot vērā rezultātus, kuri iegūti ar jauno paņēmienu, jaunais paņēmiens var būt rekomendējams plašai izmantošanai pediatriem klīnikās un ambulancēs, ātrās neatliekamās medicīniskās palīdzības ārstiem un citiem speciālistiem.

Informācijas avoti

1. Мезентериальный лимфаденит. https://www.iensmed.ru/news/post_new11294.htbl
2. Машковский М.Д. Лекарственные средства. М.:Медицина, 2 том, 1993 – 309, 327 с.
3. Alvarado A. A practical score for the early diagnosis of acute appendicitis.
4. Ohle R. The Alvarado score for predicting acute appendicitis: a systematic review.
5. Pat RU 2614723, C1, 2017, G01N33/49.
6. Гостищев В.К. Оперативная гнойная хирургия, М.: Медицина. – 1996. С.- 268.
7. Затевахин И.И. Абдоминальная хирургия (И.И.Затевахин, А.И.Кириенко, В.А.Кубышкин, Национальное руководство. Краткое описание. – М.:ГЭОТАР – Медиа, 2016 – 912 с.
8. Pat. RU 2318442, C1, 2008, A61B8/00.
9. Pat. RU 2434573, C2, 2011, A61B5/00.
10. Piet. WO 2004032711, 2004, A2, A61B1/00.
11. Piet. WO 2013075055, A2, 2013, G01N33/68.
12. Pat. RU 2684727, C1, 2019, G01N33/48.
13. https://ru.wikipedia.org/wiki/%D0%98%D0%BD%D1%82%, 01.09.2020
14. http://kalog-adm.ru/2020/07/gemoglobinemiya/, 01.09.2020
15. https://en.wikipedia.org/wili/LRG1, 01.09.2020
16. https://spravochnik.synevo.ua/ru/intro/method.html.

PRETENZIJAS

1. Gangrenoza apendicīta attīstības riska noteikšanas paņēmiens bērniem, kas raksturīgs ar to, ka akūta iekaisuma vēdera dobumā gadījumā, pirms ķirurģiskās operācijas veikšanas, izmantojot kvantitatīvo "sendviču" tipa ELISA principu, asins serumā un urīnā nosaka citokīnu daudzumu, konkrēti, interleikīnu – 6 (*IL-6*), lipokalīnu (NGAL), alfa-2-glikoproteīnu 1 (LRG1).

2. Paņēmiens saskaņā ar 1. pretenziju, kas raksturīgs ar to, ka, ja interleikīna daudzums asins serumā ir 641 pg/ml un vairāk, lipokalīna daudzums asins serumā ir 251 ng/ml un mazāk, bet alfa-2-glikoproteīna 1 daudzums asins serumā ir 81.4 ng/ml un mazāk, tad pastāv gangrenoza apendicīta attīstības risks.

Decision of the Research Ethics Committee of Rīga Stradiņš University

Veidlapa Nr. E-9 (2)

RSU ĒTIKAS KOMITEJAS LĒMUMS NR. 21 / 27.04.2017.

Rīga, Dzirciema iela 16, LV-1007
Tel. 67061596

	Komitejas sastāvs	Kvalifikācija	Nodarbošanās
1.	Profesors Olafs Brūvers	Dr.theo.	teologs
2.	Profesore Vija Sīle	Dr.phil.	filozofs
3.	Asoc.prof. Santa Purviņa	Dr.med.	farmakologs
4.	Asoc.prof. Voldemārs Arnis	Dr.biol.	rehabilitologs
5.	Profesore Regīna Kleina	Dr.med.	patalogs
6.	Profesors Guntars Pupelis	Dr.med.	ķirurgs
7.	Asoc.prof. Viesturs Liguts	Dr.med.	toksikologs
8.	Docente Iveta Jankovska	Dr.med.	
9.	Docents Kristaps Circenis	Dr.med.	

Pieteikuma iesniedzējs: **Mohits Kakars, ķirurgs**
BKUS ķirurģijas klīnika

Pētījuma nosaukums: „ Kompleksa klīniska, molekulārbioloģiska izpēte bērna vecuma akūta komplicēta apendicīta gadījumā"

Iesniegšanas datums: 27.04.2017.

Pētījuma protokols: Izskatot augstāk minētā pētījuma pieteikuma materiālus (protokolu) ir redzams, ka pētījuma mērķis tiek sasniegts iedalot pacientus vairākās grupās un veicot ar pacientiem, bez kāda apdraudējuma veselībai, drošībai un dzīvībai, klīniski-analītisku darbu (asins un citu paraugu ņemšanu, attiecīgas analīzes), iegūto datu apstrādi un analīzi, kā arī izsakot priekšlikumus. Personu (pacientu, dalībnieku) datu aizsardzība, brīvprātīga informēta bērnu vecāku (likumīgo pārstāvju) piekrišana piedalīties pētījumā un konfidencialitāte tiek nodrošināta. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Izskaidrošanas formulārs: ir

Piekrišana piedalīties pētījumā: ir

Komitejas lēmums: **piekrist** pētījumam

Komitejas priekšsēdētājs Olafs Brūvers Tituls: Dr. miss., prof.

Paraksts

Ētikas komitejas sēdes datums: 27.04.2017.

Statement from the research application register

Statiskie / Studējošā pētnieciskā darba/Nedefinēta pētījuma pieteikumu reģistrs ()

Dokuments bez satura

Dokumenta statuss

Saskaņots

Reģistrācijas numurs	SP-37/2018
Atbildīgā pētnieka uzvārds, vārds	Kakars Mohits
Pētnieka e-pasts	mohits.kakars@bkus.lv
Tālruņa Nr.	28769660
Pētījuma nosaukums	Kompleksa klīniska, molekulārbioloģiska un mikrobioloģiska izpēte bērna vecuma akūta komplicēta apendicīta gadījumā.
Pētījuma metode	1.Izveidot trīs pētījuma pacientu grupas no VSIA BKUS ārstēto pacientu vidus vecumā no 7 līdz 18 gadiem ar sekojošām diagnozēm: ķirurģiski ārstēts akūts komplicēts apendicīts (AkA), ķirurģiski ārstēts akūts nekomplicēts apendicīts (AnA), kā arī izveidot kontroles grupu. 2. Noteikt Yersinia enterocolitica antivielu līmeni asins serumā visās pacientu grupās. 3. Noteikt jaunu biomarķieru NGAL un LRG līmeni asins serumā un LRG līmeni urīnā visās pacientu grupās. 4. Veikt bakterioloģisku asins kultūras, vēdera dobuma un aklās zarnas piedēkļa mikrobioma analīzi AnA un AkA grupu pacientiem.
Darba mērķa grupa	Izveidot jaunu laboratoro biomarķieru paneli, ko iekļaut praktiskās rekomendācijās un ieviest bērnu ķirurģijas klīnikā AkA agrīnas diagnostikas algoritma veidā.
Izglītības iestāde	RSU
Norises laiks no	15.01.2018
Norises laiks līdz	01.09.2020
Saistīts ar promocijas darbu	Jā
Ētikas atļaujas numurs	Nr. 21 / 27.04.2017
Ētikas atļaujas izsniedzējs	RSU Ētikas komiteja
Atskaites datums	
Atzinums par pabeigšanu	
Ieguvumi BKUS	

Piezīmes

Saistītie dokumenti

Atvasinātie dokumenti

Atzīme par parakstu

Rezolūcija/Uzdevums

Atbildes / Jautājumi

Darbam / Izpildei

Iepazīties

Saskaņojumi / Vīzas

Nr.	Statuss	Autors	Izpildītāji	Kontrolē	Izpildīts	Termiņš	Komentāri
41234	✔	Līga Brakmane	**Dace Zavadska**		Dace Zavadska 06.04.2018 11:30	21.03.2018 - 01.04.2018	Nepieciešamas piekrišanas formas dalībai pētījumā-Pacientu piekrišanas forma!

Patient consent form used in the study

Projekta "Kompleksa klīniska, bioloģiska un mikrobioloģiska izpēte bērna vecuma akūta nekomplicēta apendicīta un akūta komplicēta gadījumā".

Skaidrojums slimnieka vecāku (aizbildņa) atļaujas saņemšanai bioloģiskā materiāla paņemšanai un izmeklēšanai

Visā pasaulē un arī Latvijā bērniem vecuma grupā no 7 līdz 18 gadiem viens no biežākajiem akūtu vēdera sāpju iemesliem ir akūts apendicīts. Ņemot vērā neķirurģiskās ārstēšanas metodes pielietošanu akūta apendicīta (AA) pacientiem bērnu vecumā, viena no svarīgākajām neatliekamās bērnu ķirurģijas problēmām ir akūta nekomplicēta apendicīta (AnA) un akūta komplicēta apendicīta (AkA) nodalīšana ārstēšanas sākuma periodā, jo AkA liecina par novēlotu diagnostikas procesu un prasa tikai neatliekamu ķirurģisku ārstēšanu. Šodien eksistējošās AkA diagnostikas problēmas rada nepieciešamību meklēt jaunus agrīnās diagnostikas indikatorus pacientiem bērna vecumā, lai samazinātu komplikāciju attīstības biežumu, kā arī novērstu letalitātes risku.

Laika posmā no 2017. līdz 2020. gadam VSIA BKUS paredzēts veikt prospektīvu, randomizētu pētījumu, kurā iekļaus bērnus vecumā no 7 līdz 18 gadiem ar akūtu apendicītu. Tiks veikta klīniska, bioloģiska un mikrobioloģiska izpēte, sniedzot ieguldījumu patoloģijas etiopatoģenēzē un radot kritērijus akūta nekomplicēta un akūta komplicēta apendicīta agrīnai diagnostikai.

Tiks veidotas 3 pacientu grupas, katrā grupā iekļaujot 30 pacientus, t.s. 1 kontroles grupa (K). Pacientu ārstēšanu nodrošinās VSIA BKUS Bērnu ķirurģijas klīnikā praktizējoši sertificēti bērnu ķirurgi.

Visiem pacientiem pirms iekļaušanas pētījumā tiks veikts AA skrīnings. AA skrīningā bez klīniskā stāvokļa novērtējuma tiks iekļauti sekojoši objektīvi kritēriji: pilna asins aina (PAA), C-reaktīvais olbaltums (CRO), interleikīns-6 (IL-6) un vēdera ultrasonogrāfija (USG). Iekļautajiem pacientiem tiks veikta asins seruma un urīna paraugu paņemšana biomarķieru *NGAL* (serumā) un *LRG* (serumā un urinā) noteikšanai. Tāpat

tiks veikta *Yersinia enterocolitica* asins seroloģija. AnA un AkA grupu pacientiem pirms operācijas tiks iegūta asins kultūra.

Operācijas laikā tiks paņemta mikrobioloģiskā kultūra no pacienta vēdera dobuma un aklās zarnas piedēkļa lūmena proksimālā un distālā gala. Pēc tam aklās zarnas piedēklis tiks nosūtīts histoloģiskai izmeklēšanai. Balstoties uz atradi operācijas laikā, pacienti tiks iedalīti AkA un AnA grupā, t.i. tiks pabeigta pacientu randomizācija. AkA un AnA grupu pacientiem tiks veikti asins seruma un urīna paraugu paņemšana biomarķieru *NGAL* (serumā) un *LRG* (serumā un urīnā) noteikšanai arī 2.un 5. pēcoperācijas dienā.

Izmeklējumi slimniekam, slimnieka vecākiem (aizbildnim) būs bezmaksas. Tāpat slimnieks, slimnieka vecāki (aizbildnis) nesaņems finansiālu atlīdzību par izmeklējumu veikšanu. Tiks ievēroti Helsinku deklarācijas pamatprincipi un pacientu datu aizsardzības likuma prasības. Pēc slimnieka vecāku (aizbildņa) vēlēšanās tiks sniegta informācija par iegūtajiem izmeklējumu rezultātiem.

Pētījuma mērķu un uzdevumu realizācija neapdraud pacientu drošību. Slimnieki tiks informēti par pētījuma mērķi un bioloģiskā materiāla paņemšanas veidu.

Gadījumos, ja slimnieks, slimnieka vecāki (aizbildnis) nepiekritīs izmeklējumu veikšanai, izmeklējumi netiks veikti. Atteikums neietekmēs uzsāktās ārstēšanas apjomu un kvalitāti.

Ar nepieciešamo izmeklējumu būtību esmu iepazīstināts un piekrītu, ka manam bērnam slimības diagnostikas un ārstēšanas etapos tiks paņemts un izpētīts bioloģisks materiāls.

Pacients …….. (vārds, uzvārds)

Pacienta personas kods ……..

Pacienta paraksts……..

Pacienta vecāku (aizbildņa) …… (vārds, uzvārds)

Pacienta vecāku (aizbildņa) personas kods: ……..

Pacienta vecāku (aizbildņa) paraksts: ……(atšifrējums)

Datums:

Projekta vadītāja paraksts: ……
(atšifrējums)

Yersinia antibody request form

E. Gulbja Laboratorija
Brīvības gatve 366, Rīga, LV-1006, Latvija
Tel. 67801054, fax 67543867, e-mail: info@egl.lv

Nosūtījuma veidlapa		
Pētnieks:	**Prof. Arnis Eņģelis**	(ENGAR)
Maksātājs:	**Rīgas Stradiņa universitāte**	(JERSU)
! Atzīmēt šādi: ☒		
Pacients:	Vārds, uzvārds vai pacienta numurs _____	
	Dzimšanas datums: __ __ __ __ __ __	
	Dzimums: ☐ vīrietis ☐ sieviete	
Analizējamais materiāls ņemts:	Datums: __ __. __ __. __ __ __ __	Laiks: __ __ : __ __
Testēšanas pārskatu saņēmēji:	☒ Prof. Arnis Eņģelis	(ENGAR, EMAIL)
	☒ Dr. Mohits Kakars	(KAKMO, EMAIL)
Mikrobioloģijas analīzes		
6305	☐ Jersīniju antivielas	

Pētnieks: _____

(Paraksts un tā atšifrējums/zīmogs)