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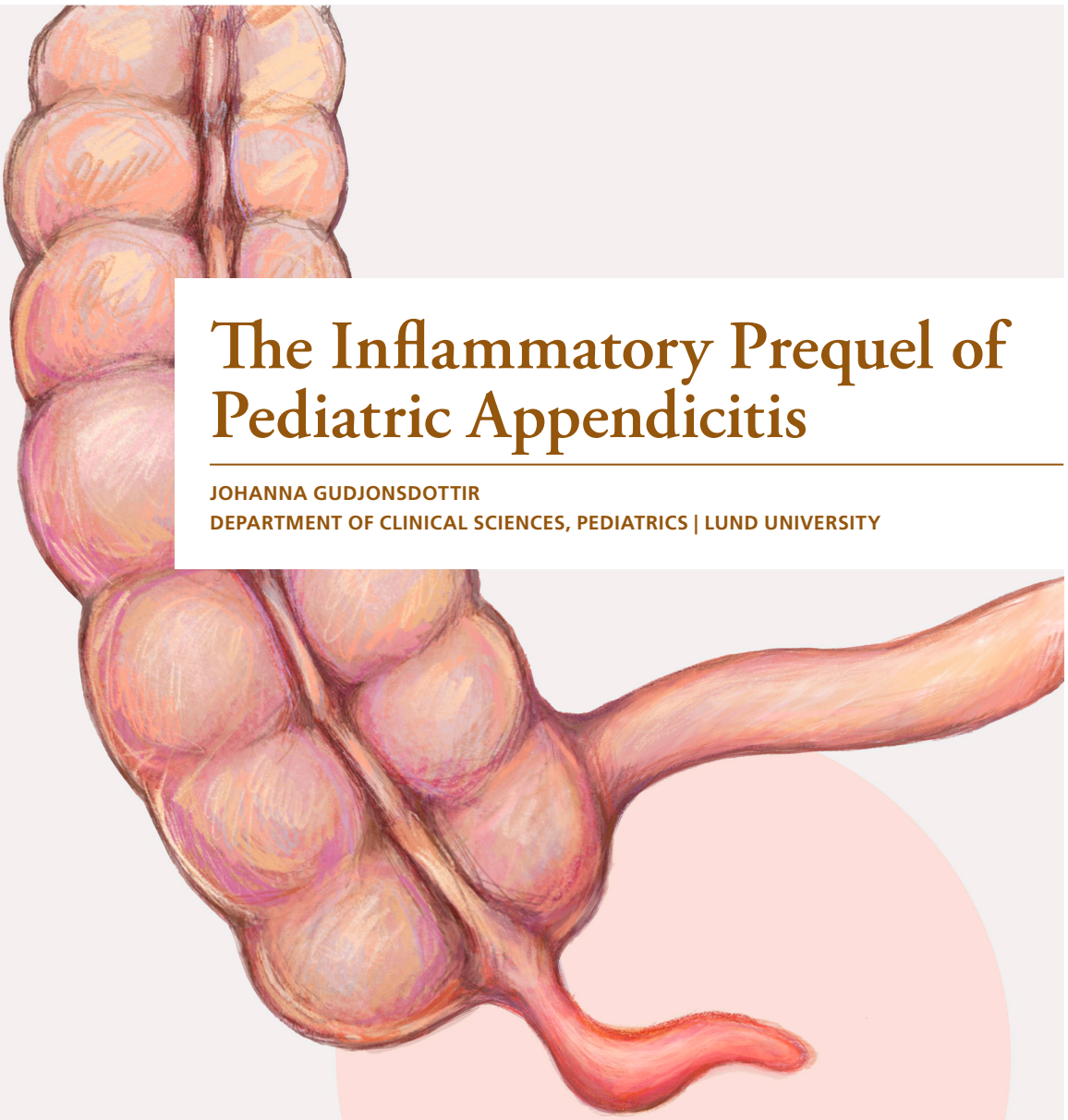
2022

Document Version:
Publisher's PDF, also known as Version of record



The Inflammatory Prequel of Pediatric Appendicitis

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LUND
UNIVERSITY

DOCTORAL DISSERTATION
by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at LUX B152, Helgonavägen 3, Lund, Sweden,
on the 22nd of April 2022, 1.00 pm.

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Associate Professor Martin Salö

Organization	Document name
LUND UNIVERSITY	**DOCTORAL DISSERTATION**
	Date of issue **23 APRIL 2022**
Author **JOHANNA GUDJONSDOTTIR**	Sponsoring organization

Title

THE INFLAMMATORY PREQUEL OF PEDIATRIC APPENDICITIS

Abstract

Background: Appendicitis is the most common disease requiring acute abdominal surgery in children, yet the pathogenesis of appendicitis is not fully understood. It can be challenging to diagnose appendicitis clinically, especially in young children, leading to high rates of initial misdiagnosis. It is not clear why some children are affected by a complicated disease course while others recover spontaneously. It has been proposed that different immune responses in different individuals propel the inflammation towards an uncomplicated or a complicated disease course. To date, there is no reliable measure to distinguish between patients with uncomplicated appendicitis and those with complicated appendicitis.

Aims: To increase the knowledge of how the inflammatory processes anteceding pediatric appendicitis can be categorized, modulated, and detected.

Methods: Papers I, IV and V were prospective clinical institution-based studies. Paper II was a retrospective institution-based cohort study and Paper III a nationwide cohort study. In Paper I the diagnostic performances of four different clinical prediction scores for pediatric appendicitis were evaluated. In Papers II and III the associations of immunoglobulin E (IgE)-mediated allergy and complicated appendicitis in children were evaluated. In Paper IV we assessed the associations of biological stress, measured as hair cortisol concentrations (HCC) and pediatric appendicitis. In Paper V the associations of serum concentrations of IgE and T helper cell 2 (Th2)-associated cytokines with complicated appendicitis were evaluated.

Results: The clinical prediction scores appendicitis inflammatory response (AIR) score and pediatric appendicitis risk calculator (pARC) displayed significantly higher specificity and positive predictive value and lower rates of negative appendectomies compared to the pediatric appendicitis score (PAS) and Alvarado score (I). Children with IgE-mediated allergy had a significantly reduced odds of complicated appendicitis (aOR 0.33 [95% CI 0.18-0.59], p<0.001 (II), and aOR 0.80 [95% CI 0.67-0.96], p=0.021 (III)). The risk of complicated appendicitis among allergic children was reduced by one-third compared to that in non-allergic children (IR 0.13 vs 0.20 per 1000 person-years, HR 0.68 [95% CI 0.58-0.81], p<0.001), while the risk of uncomplicated appendicitis did not vary with allergy status (IR 0.91 vs 0.91, HR 1.00 [95% CI 0.94-1.07], p=0.932). Seasonal antigen exposure was a protective factor for complicated appendicitis (aOR 0.82 [95% CI 0.71-0.94], p=0.004), and ongoing antihistamine medication was a risk factor (aOR 2.28 [95% CI 1.21-4.28], p=0.012) (III). An increase in HCC was associated with an increased risk of appendicitis (aOR 10.76 [95% CI 2.50-46.28], p=0.001) and complicated appendicitis (aOR 7.86 [95% CI 1.20-51.63], p=0.03) (IV). High concentrations of IL-13 were associated with an increased risk of complicated appendicitis (aOR 1.02 [95% CI 1.01-1.04], p=0.011). Serum concentrations of IgE, IL-4, and IL-9 were not significantly associated with the risk of complicated appendicitis (V).

Conclusions: AIR score and pARC are superior to the PAS and Alvarado score for diagnosing appendicitis in children. Children with allergy have a lower risk of complicated appendicitis, but the same risk of uncomplicated appendicitis, compared to non-allergic children. Increased stress, measured as an increase in HCC, is associated with an increased risk of appendicitis and complicated appendicitis in children. High levels of IL-13 seem to be associated with an increased risk of complicated appendicitis in children.

Key words

Allergy, appendicitis, clinical prediction scores, immunoglobulin E, inflammation, stress

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language **ENGLISH**
ISSN and key title **1652-8220**		ISBN **978-91-8021-223-6**
Recipient's notes	Number of pages **100**	Price
	Security classification	

Signature J. Gudjonsdottir Date 2022-03-08

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Cover illustration by Julia Hallström Hjort
Author portrait by Nicole Gustavsson
Illustrations in the thesis by the author

Faculty of Medicine
Department of Clinical Sciences, Pediatrics

ISBN 978-91-8021-223-6
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

Till mina föräldrar, Annette and Elvar

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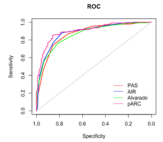
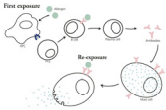
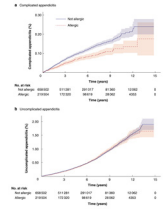
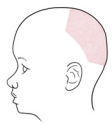
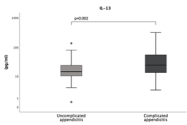
V. **An Evaluation of Serum IgE and Th2-associated Interleukins in Children with Uncomplicated and Complicated Appendicitis**
Gudjonsdottir J, Roth B, Lovén G, Ohlsson B, Hagander L, Salö M
Submitted manuscript

Thesis at a glance

	Aim	Method	Main results	Teaser	Conclusion
I	To evaluate four clinical prediction scores for children with suspected appendicitis.	Prospective cohort study of 318 children with suspected appendicitis. Diagnostic evaluation and comparison of the PAS, Alvarado score, AIR score and pARC.	The AIR score and the pARC had higher specificity, positive predictive values, and net benefit value than the PAS and Alvarado score. Sensitivity and negative predictive values were similar for all prediction scores. All prediction scores had high AUROC values.		The AIR score and pARC have a higher overall diagnostic accuracy. Safely ruling out appendicitis based on clinical prediction scores is a challenge.
II	To evaluate the risk of complicated appendicitis in children with IgE-mediated allergy.	Retrospective cohort study of 605 children who had undergone appendectomy.	Complicated appendicitis occurred in 20 children with allergy (19.6%) and in 236 with no allergy (46.9%), aOR 0.33 (95% CI 0.18-0.59), p<0.001.		Children with IgE-mediated allergy have a lower risk of complicated appendicitis compared to non-allergic children.
III	To compare the relative and absolute risk of complicated appendicitis in allergic and non-allergic children, and if it was modified by season, allergy medication and timing of allergy diagnosis.	Nationwide cohort study including all children born in Sweden between 2000 and 2010. Cross-sectional and longitudinal analysis.	Allergic children had a lower adjusted odds for complicated appendicitis (aOR 0.80 (95% CI 0.67-0.96), p=0.021). HRs for complicated and uncomplicated appendicitis in allergic children were 0.68 (95% CI 0.58-0.81), p<0.001) and 1.0 (95% CI 0.94-1.07), p=0.932), respectively.		Children with IgE-mediated allergy have a lower risk of complicated appendicitis but the same risk of uncomplicated appendicitis as non-allergic children. Antigen exposure is a protective factor and antihistamine treatment a risk factor.
IV	To investigate the associations of stress with appendicitis and complicated appendicitis in children.	Prospective case-control study including 51 children treated for appendicitis and 82 controls. Hair cortisol concentrations reflecting the activity of the HPA axis in months 0-3 and 4-6 were measured.	Increased hair cortisol concentrations were associated with an increased risk of appendicitis (aOR 10.76 (95% CI 2.50-46.28), p=0.001) and complicated appendicitis (aOR 7.86 (95% CI 1.20-51.63), p=0.03).		Increased stress, measured as an increase in hair cortisol concentrations, is associated with an increased risk of appendicitis and complicated appendicitis in children.
V	To evaluate the associations of Immunoglobulin E and Th2-associated interleukins with complicated appendicitis in children.	Prospective cohort study of 178 children with suspected appendicitis. Evaluation of associations between serum concentrations of total IgE and IL-4, IL-9, and IL-13 with the risk of complicated appendicitis.	High concentrations of IL-13 were associated with an increased risk of complicated appendicitis (aOR 1.02 (95% CI 1.01-1.04), p=0.01). Serum concentrations of IgE, IL-4 and IL-9 did not significantly affect the risk of complicated appendicitis.		High concentrations of serum IL-13 are associated with an increased risk of complicated appendicitis in children.

Abbreviations

ACTH	Adrenocorticotropic hormone
AIR score	Appendicitis inflammatory response score
ANC	Absolute neutrophilic count
APC	Antigen presenting cell
ATC	Anatomical therapeutic chemical
AUC	Area under the curve
CD	Crohn's disease
CI	Confidence interval
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CT	Computed tomography
ED	Emergency department
GALT	Gut-associated lymphatic tissue
HCC	Hair cortisol concentrations
HPA axis	Hypothalamic-pituitary-adrenal axis
HR	Hazard ratio
ICD	International classification of diseases
ICS	Inhalation corticosteroid
IFN-γ	Interferon gamma
IgE	Immunoglobulin E
IL	Interleukin
IQR	Interquartile range
IR	Incidence rate
LA	Laparoscopic appendectomy
LRG	Leucine-rich alpha-2-glycoprotein
MRI	Magnetic resonance imaging
MSD	Mesoscale Discovery®

NK cell	Natural killer cell
NOM	Non-operative management
NPV	Negative predictive value
OA	Open appendectomy
OR	Odds ratio
pARC	Pediatric appendicitis risk calculator
PAS	Pediatric appendicitis score
PMN	Polymorphonuclear leukocytes
PPV	Positive predictive value
RCT	Randomized controlled trial
ROC	Receiver operating characteristics
SD	Standard deviation
Th cell	T helper cell
TMB	Tetramethylbenzidine
TNF-α	Tumor necrosis factor alpha
UC	Ulcerative colitis
US	Ultrasound
WBC	White blood cell count

Introduction

The appendix

A historical résumé

The function of the appendix as well as the etiology and pathogenesis behind appendicitis are poorly understood, and yet humans have been interested in the organ since at least the time when the pyramids were built, roughly 2500 years BC. During the mumification process popular at the time, all the viscera were removed from the body and placed into separate jars. Some of these jars had inscriptions on their exterior referring to the "worm of the bowel" – probably referring to the appendixes[1]. In 30 years AD, Aretaeus of Cappadocia, a physician in ancient Greece, documented that he had treated a patient suffering from an abscess on the right side of the colon by incising it, whereafter the patient recovered. This has later been considered the oldest documented drainage of an appendiceal abscess[2]. Neither of the two prominent anatomical works of Aristotle (4th century BC) and Galen (2nd century AD) mention the appendix, probably since they primarily dissected animals that do not have appendixes[1].

The oldest preserved documentations of the appendix in Western European literature appeared in the renaissance period. In 1492, the appendix was included in anatomical drawings of Leonardo da Vinci. It was also well depicted in the famous work "De Humani Corporis Fabrica", published in 1543 by Andreas Vesalius[3]. The first written descriptions were made by the Italian Berengario da Carpi in 1524. However, at that time nothing was known of the significance of the pathology and physiology of the appendix[1].

The first description of appendicitis was attributed in 1544 to Jean Fernel when, during an autopsy, he found that both the appendix and cecum of the deceased patient were necrotic and had perforated. During the following two centuries, appendicitis was diagnosed first and foremost post-mortem. During this time the origin of the inflammation in the right lower quadrant was a very controversial topic. In the beginning of the 19th century, the leading medical profession believed that the inflammation originated from the cecum ("typhlitis") or the connective tissue surrounding it ("perityphlitis"), rather than from the appendix. Despite repeated observations of the appendix as the primary focus of inflammation, this was hard to accept, possibly due to the lack of therapeutic implications. At this time point, general

anesthesia had not yet been introduced, and surgery was primarily a last resort in the treatment of appendicitis. The first ever procedure under general anesthesia was performed in 1846[4].

In 1735, the first documented appendectomy was performed by the French surgeon Claudius Amyand. The patient, an 11-year-old boy, had a long-standing scrotal hernia with a fecal fistula to the thigh. When Amyand opened the hernia sac, he found that the appendix had been perforated by a pin, giving rise to the fistula. The appendix was removed surgically, and the fistula opened, whereafter the patient recovered[5]. Hence, the first-known appendectomy was performed through the sac of an inguinal hernia. Thanks to his discovery, the presence of the appendix in an inguinal hernia is called Amyand's hernia[6] – a rather rare condition occurring in around 0.4%-0.6% of all inguinal hernias. Appendicitis within the sac of an inguinal hernia is even rarer, occurring only in around 0.1% of all Amyand's hernia cases[7].

In 1886, Reginald H Fitz presented his paper "Perforating Inflammation of the Vermiform Appendix: With Special Reference to Its Early Diagnosis and Treatment", in which he clearly stated that inflammation in the right lower quadrant begins in the appendix. He also advocated early surgical treatment, with removal of the appendix[8]. Fitz was the first to use the term "appendicitis" – a word that has been criticized due to its construction of a Latin stem and Greek suffix. Nonetheless, it became widely accepted. From here on, the theories of "typhlitis" and "perityphlitis" were gradually rejected.

In 1889, the American surgeon Charles McBurney published the first of many important studies on appendicitis[9]. He has described the point of most tenderness in appendicitis, now known as McBurney's point, and the lateral muscle-splitting or "gridiron" incision, now known as McBurney's incision[10].

Despite the introduction of early appendectomy for acute appendicitis, the mortality rate remained high, especially in patients with generalized peritonitis. Through a better understanding of the mechanisms underlying peritonitis, the development of anesthesia and perioperative care, and finally after the introduction of antibiotics, the mortality rate has decreased.

In 1981, the German gynecologist and laparoscopic pioneer Kurt Semm performed the first laparoscopic appendectomy. This did not go down well among many of his peers: the president of the German Surgical Society advocated for the revocation of Semm's medical license, and the American Journal of Gynecology and Obstetrics refused to publish his paper on laparoscopic appendectomy since they considered the described method as "unethical"[11,12]. Laparoscopic appendectomy in children was first described in 1992[13].

"The history of appendicitis includes examples of great resistance to changing concepts, brilliant but unaccepted early observations, emotional support for unsupportable views, the importance of timing, and, finally, the development of a highly satisfactory solution."

G Rainey Williams,
Presidential Address: A history of appendicitis,
Annals of Surgery (1983)

Embryology

Most of the intestines, including the cecum and the appendix, are developed from the embryonic midgut. During the 6th week of gestation, the midgut bends around the superior mesenteric artery, forming what is called the "midgut loop"[14]. At the same time, the primordium of the cecum and appendix appears as a cecal bud on the antimesenteric border of the caudal midgut loop[15]. The cecal bud grows at the same pace as the rest of the colon, while the lower part lags behind and forms a distinct entity from the rest of the cecum. The appendix then elongates rapidly and becomes visible during the 8th week of gestation. At first, the appendix is located at the apex of the cecum, but as the right haustra of the cecum grows, it is translocated medially and cranially. During the 14th gestational week, lymphatic tissue begins to develop in the appendix[14].

Anatomy

The appendix is a tubular blind-ended structure protruding from the posterior medial part of the cecum, in general 2 to 5 cm below the ileocecal valve. Based on examinations of 210 appendixes without histopathological signs of inflammation from children aged 0-17 years, it seems that the appendix grows from infancy to the age of 3 years, by which time it reaches its final proportions of approximately 6.5 mm in diameter and 66.5 mm in length[16]. The positioning of the tip of the appendix can vary greatly, including retrocecal (most common), pelvic, subcecal, preileal and postileal (Figure 1)[17,18].

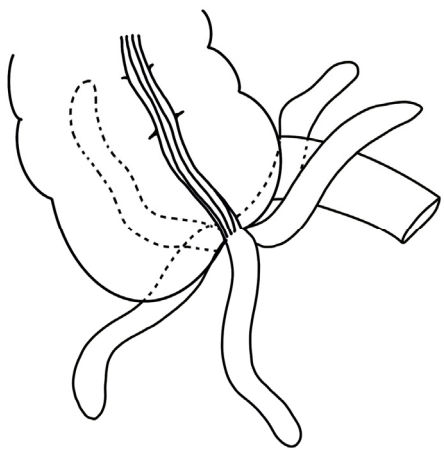


Figure 1. An illustration of the various positions of the appendix in relationship to the cecum and the distal ileum.

The appendix is attached to the ileum and cecum by the mesoappendix, a peritoneal fold containing blood and lymphatic vessels as well as nerves. Blood supplies the appendix through the appendicular artery, a small branch of the ileocolic artery which, in turn, originates from the superior mesenteric artery.

The appendix is drained venously through the appendicular, ileocolic, and superior mesenteric veins. The superior mesenteric vein combines with the splenic vein behind the neck of the pancreas to form the hepatic portal vein.

The lymphatic drainage runs parallel to the arteries. The visceral peritoneum contains sympathetic innervation from the celiac and superior mesenteric ganglia, while the parietal peritoneum is innervated by somatic sensory nerve fibers entering at level Th10.

Congenital malformations of the appendix are extremely rare. Among them are the total absence (agenesis)[19] and duplication[20] of the vermiform appendix. Another rare malformation is horseshoe appendix[21], in which both ends of the appendix are attached to and communicate with the cecum.

Histology

The appendiceal wall contains the same layers as the rest of the intestinal canal: mucosa, submucosa, muscularis externa and serosa. The muscularis externa consists of two layers of smooth muscle: an inner circular and an outer thin longitudinal layer. The appendix distinguishes itself from the rest of the intestine through its abundance of lymphoid tissue with a lymph node-like structure[22]. Along with Peyer's patches of the ileum and isolated lymphoid follicles, the appendix constitutes the human gut-associated lymphoid tissues (GALT)[23].

Function

In rabbits, for example, the appendix is substantially larger than in humans and is involved in the digestion of cellulose. The human appendix has long been considered a rudimentary part of the intestines: a part of the cecum that shrunk when our diet changed. However, this outlook is being reconsidered as more recent evidence suggests that the human appendix is involved in interacting with and handling intestinal microorganisms[24].

The appendix has been shown to interact with the intestinal flora and seems to act as a reservoir or "safe-house" for the commensal gut microbiome[25,26]. The mucosa of the large intestine and the appendix is lined with a biofilm consisting of commensal gut bacteria in a mucosal matrix which is thought to prevent pathogens from crossing the

intestinal barrier, but also to harbor non-pathogenic commensal bacteria. When the colonic biofilm is disturbed or flushed out as a response to an infection, the normal gut flora is thought to be restored through shedding of the biofilm and re-inoculation of beneficial bacteria from the appendix to the proximal colon[25]. The appendix might also serve as a priming site for immune cells, along with the other GALT sites[23]. Furthermore, the appendix secretes immunoglobulin (Ig) A, which neutralizes and facilitates elimination of intestinal pathogens[27].

With modern hygiene and medicine, these functions do not seem essential, but appendectomy has been found to be associated with more severe *Clostridium difficile* infections[28].

Appendicitis

Appendicitis is the most common disease requiring emergency abdominal surgical intervention in children[29]. In 2020, a total of 12,922 appendectomies were performed in Sweden, 1878 of these on patients younger than 15 years[30]. This yields an incidence of about 120 cases per 100,000 persons in 2020[30].

Epidemiology

Appendectomy is the most frequently performed acute abdominal operation worldwide[31]. The lifetime risk of appendicitis has been estimated to be 8.6% for men and 6.7% for women[32]. Appendicitis is uncommon in young children, but the incidence peaks during the second or third decades of life[32]. Although the incidence of appendicitis is higher in males, the lifetime risk of appendectomy is 12% for men and 23% for women[32]. A systematic review from 2017 has shown that the incidence of appendicitis in the Western world peaked around the 1950s, to then decline during the latter half of the 20th century. During the 21st century, the incidences of both non-perforated and perforated appendicitis have plateaued, while the incidence of appendectomy has decreased steadily, probably due to fewer negative appendectomies[33]. However, in newly industrialized countries, the incidence of appendicitis is increasing steadily, mirroring the increase in the Western World in the early 20th century[33]. To date, the incidences of appendicitis in some parts of Asia, the Middle East and South America are higher than in many Western countries. Based on these findings, it has been suggested that the etiology of appendicitis, at least to some extent, is associated with multifactorial environmental changes concurrent with

industrialization[33]. It is, however, also possible that an increased health care coverage leads to increased diagnosis of appendicitis.

The incidence of appendicitis among Swedish children has also declined during the last decades, mainly represented by a decrease in the numbers of non-perforated appendicitis along with the reduction of negative appendectomies[34].

Appendicitis is, as previously mentioned, more common in adolescents than in adults[32]. It is, however, less common in young children, especially those under 5 years of age[35–37]. The frequency of complicated appendicitis varies but is higher in younger patients[38]. In a study from the USA the frequency of complicated appendicitis was 39.7% in children aged 0-9 years and 25.1% in children aged 10-19 years[39]. Another American study reported a perforation frequency of 29.7% in all age groups[40].

Epidemiological studies have shown a seasonal pattern in the incidence of appendicitis, with most peaks occurring during the summer months[40–42]. The reasons for this remain unclear[43].

Etiology

Even though a couple of hundred years have passed since the first appendectomy was performed, the etiology and pathogenesis of appendicitis are still not understood completely.

Obstruction

The most accepted theory is that appendicitis is caused by an obstruction of the appendix lumen (Figure 2). This theory was initially based on the frequent findings of appendicoliths (hard fecal masses with mineral deposits) in the most advanced cases of appendicitis. Experimental studies on animals[44,45] and humans[46] strengthened this theory scientifically by showing that when the appendicular lumen is obstructed completely by ligation or a balloon catheter, appendicitis will result. The proposed mechanism is that obstruction by an appendicolith, lymphatic hyperplasia, or (more uncommonly) a foreign body or a neoplasm, will lead to subsequent accumulation of secretions, an increase in luminal pressure, impaired venous and lymphatic drainage, an increased mucosal permeability and finally overgrowth and invasion of bacteria. However, when the luminal pressure was measured perioperatively during appendectomy in 24 patients, only five out of a total of 19 cases of appendicitis (three out of three with gangrenous appendicitis and two out of 16 with phlegmonous appendicitis) had an increased luminal pressure. The authors then suggested that the appendiceal obstruction with an increased intraluminal pressure is a consequence of the

inflammation itself and may be associated with the development of complicated appendicitis[47]. When the prevalence of appendicoliths has been studied, they have been found in a wide variety of appendicitis cases (6%[48], 13.7%[49], 23-52%[50]), but also in patients with healthy appendixes[50,51]. In a study of 1711 appendixes with appendicitis, lymphoid hyperplasia was present only in 15 cases[48]. Hence, it seems that obstruction, primarily by an appendicolith or lymphoid hyperplasia, is unlikely to be the primary cause of most of the cases of appendicitis[49,52]. It should, however, be noted that the presence of an appendicolith is associated with an increased risk of complicated appendicitis[53,54]. Even though it is now clear that a luminal obstruction cannot explain all cases of appendicitis, this mechanism is still taught in several medical textbooks.

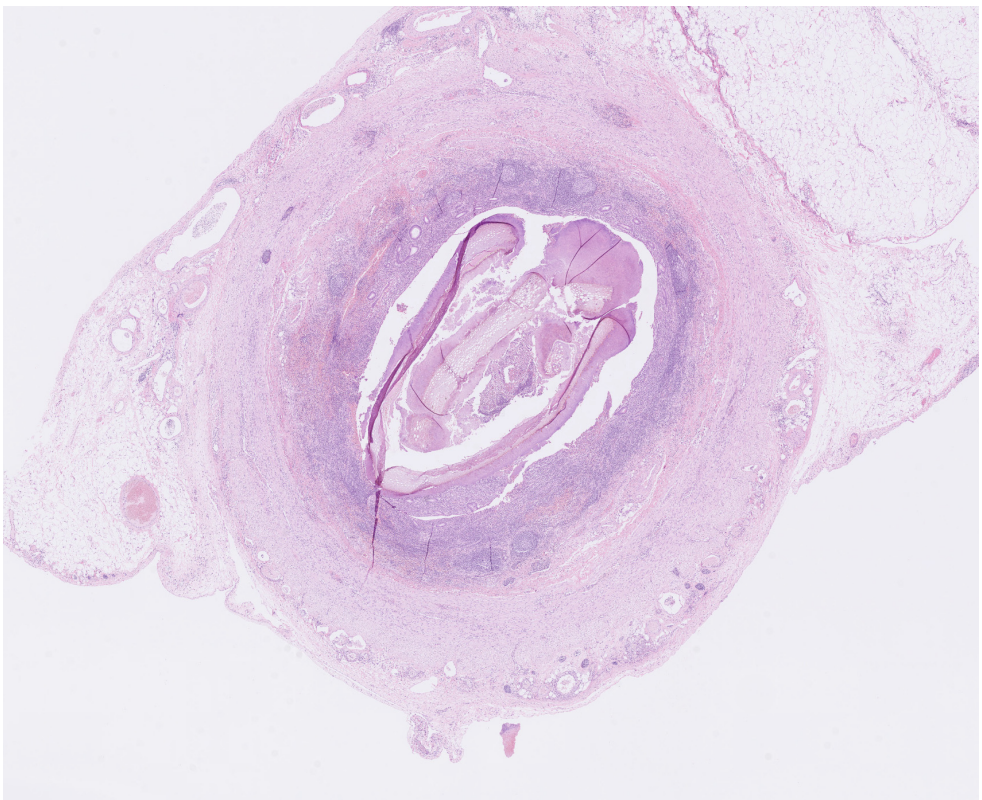


Figure 2. A case of phlegmonous appendicitis with vegetables in its lumen. Image from the Department of Pathology, Skåne University Hospital, Lund, Sweden.

Infection

The occurrence of appendicitis in space and time clusters has raised the question of an infectious etiology, but no specific bacteria or viruses have been identified[43,55].

Genetics

Several papers have reported an increased risk of appendicitis in individuals with relatives who have had the disease[56,57]. A review article has proposed that polygenetic inheritance might explain half of the variance in the risk of appendicitis and has found that a positive family history increases the relative risk of appendicitis by three-fold[58]. It should be noted that many studies investigating hereditary or familial effects often study the risk of appendectomy and that social and behavioral factors can affect that risk[59]. Hypothetically, patients with a familial history of appendicitis might be more inclined to seek medical care due to abdominal pain.

A Swedish study of twins found that non-shared environmental factors accounted for most of the variability of risk for appendicitis, and only a small (30%) genetic effect. In males, almost no genetic effects were found[60]. Some studies have reported associations between appendicitis and specific genes[61,62].

Other causes

Even though rare, appendicitis can be induced by blunt abdominal trauma[63,64]. There have also been reports of appendicitis caused by migrating gallstones[65]. Furthermore, it has been suggested that low fiber intake could be part of the pathogenesis of appendicitis[66,67].

Classifications

The diagnosis of appendicitis is usually confirmed by the perioperative findings. Since a discordance between the clinical and the histopathological diagnoses of the different grades of inflammation is common[68,69], the histopathological diagnosis is considered gold standard in clinical studies. But unfortunately, different pathologists may disagree in their assessments[70].

Catarrhal appendicitis

This is inflammatory infiltration of neutrophils confined to the mucosa. The clinical relevance of this finding has been disputed, since it is found frequently in patients without clinical symptoms of appendicitis who have undergone incidental appendectomies[71]. Mucosal inflammation alone should not be regarded as true appendicitis, and other causes of the patient's symptoms should be considered.

Phlegmonous appendicitis

Phlegmonous appendicitis (Figure 3) is defined as a transmural inflammation with infiltration of neutrophils through the mucosa, submucosa and muscularis propria. The

mucosa is usually ulcerated, and findings of edema, fibrinopurulent serositis, micro-abscesses in the appendiceal wall and small vascular thrombi are common[52].

Gangrenous appendicitis

Gangrenous appendicitis includes the inflammatory changes of phlegmonous appendicitis, but with a cardinal feature of full-thickness necrosis of the appendicular wall. This can be seen macroscopically as a gray or black discoloration of the appendix. If left untreated, it will most likely eventually progress to perforation[52].

Perforated appendicitis

The histopathology of perforated appendicitis is the same as for gangrenous appendicitis, with the addition of a visual hole in the appendicular wall or the perioperative finding of an appendicolith in the abdominal cavity[72].

Appendicular abscess

An appendicular abscess includes perforated appendicitis with encapsulated pus around the appendix or in other locations in the abdomen, for example in the pouch of Douglas.

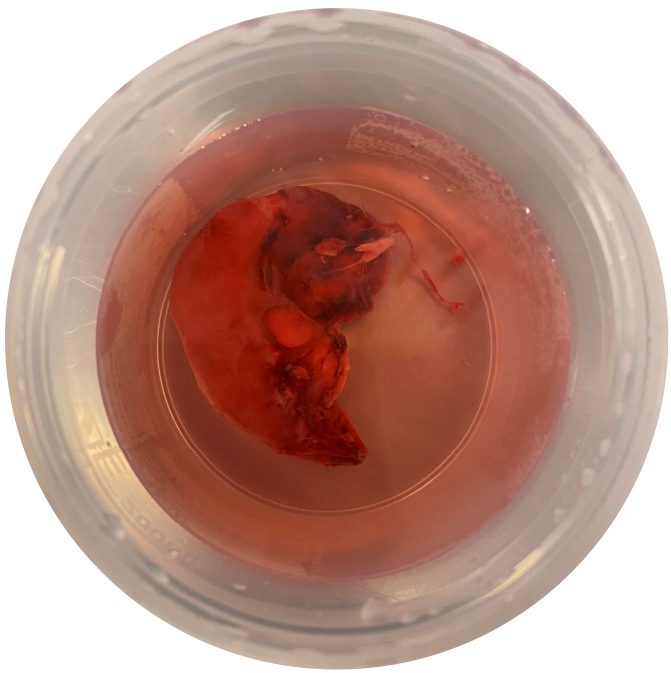


Figure 3. A macroscopically phlegmonous appendix in a jar with formalin. Diagnosis was confirmed through histopathological examination. My first laparoscopic appendectomy as a surgical resident, Malmö, Sweden, November 2020.

Natural course of appendicitis

Appendicitis has long been considered a progressive disease, always leading to perforation if left untreated. This has created a widespread tradition of emergency appendectomy with minimal delay, and with it an acceptance of relatively large numbers of negative appendectomies. The approach with early exploration to prevent perforation was adopted over a 100 years ago, when appendicitis was still a considerable cause of mortality. Since then, the mortality of appendicitis has decreased dramatically, and the view of appendicitis as a homogenous progressive disease has been increasingly challenged. It is now evident that far from it being the case that all incidences of appendicitis will eventually perforate if left untreated, many will even resolve spontaneously[73].

Already in 1964 Howie compared the outcomes of surgical units with different approaches to cases of suspected appendicitis[74]; they were either "radical", meaning that they liberally performed appendectomies, or "conservative", meaning that they abstained from surgery if they assessed that the patient's symptoms could subside spontaneously. Not surprisingly, the conservative units performed fewer negative appendectomies. However, the conservative units also treated fewer patients with true appendicitis (transmural inflammation).

Thirty years later, Andersson et al confirmed these results, showing that the rate of appendectomy does not influence the incidence of perforating appendicitis, but the incidence of non-perforating appendicitis[75]. They assumed that a readiness to explore patients with suspected appendicitis increased the number of confirmed cases by adding patients whose inflammation would otherwise resolve spontaneously and thereby pass undetected.

A more expectant or "conservative" approach to patients with suspected appendicitis leads to fewer cases of non-perforating appendicitis and fewer negative appendectomies but does not increase the incidence of perforating appendicitis. The variance in appendectomy incidence of different surgical centers is hence thought to explain the difference in perforation rates. Resolution of appendicitis has been described extensively in both clinical and radiological studies[76–78], as well as in histological studies[79].

Andersson further described the alternative natural course of appendicitis in 2007, concluding that many cases of non-perforated appendicitis resolve spontaneously and only a small proportion advance to perforation. Based on this, a focus shift in the management of suspected appendicitis was advocated, from prevention of perforation to correct diagnosis and early treatment of complicated cases[73]. Another study from the same year also reported a disconnection in trends between non-perforated and

perforated appendicitis, which would not be expected if perforated appendicitis were simply the result of delayed treatment[80].

Uncomplicated and complicated appendicitis – separate disease entities driven by different immune responses?

Due to the heterogenicity of the natural course of appendicitis, the condition is usually categorized as either uncomplicated or complicated. Uncomplicated appendicitis can resolve spontaneously but might reoccur, while complicated appendicitis can progress rapidly with gangrene and perforation. An understanding of what causes the disease to progress towards self-healing contra perforation is crucial for surgeons to correctly diagnose and treat the complicated cases appropriately, while deferring from emergency surgical treatment of cases with a low risk of perforation.

Among other factors, it has been suggested that the disease trajectory is, at least in part, dependent on the individual's immunological response. This theory was based primarily on the findings of a positive association of appendectomy and the T helper cell (Th)1-associated Crohn's disease (CD)[81] and an inverse association of appendectomy at a young age and the Th2-associated ulcerative colitis (UC)[82].

It is important to note that follow-up studies on appendectomy and CD have reported inconsistent results, and some have indicated that the increased risk of CD after appendectomy is transient and may reflect a diagnostic challenge in patients with beginning CD[83–85]. The lower risk of UC after appendectomy at a young age, however, was also found among first-degree relatives, indicating shared genetic or inflammatory factors increasing the risk of appendicitis while decreasing the risk of UC[86].

Furthermore, the incidence of appendicitis is lower during pregnancy, especially in the third trimester[87–89]. A rebound effect with an increased risk of appendicitis lasting up to 2 years postpartum has been reported[89,90]. This suggests a protective effect of pregnancy on appendicitis, which might be attributed to alterations in female sex hormone levels and/or a selective down-regulation of the immune system[88]. Pregnancy leads to complex changes in the maternal immune system to create a tolerance toward fetal antigens without compromising the protection from true pathogens. Among these changes, an upregulation of the Th2-mediated immunity has been suggested[91].

Clinical studies have found associations of gangrenous appendicitis and the production of Th1- and Th17-associated cytokines, further supporting the theory of immune-modulating effects on the disease course of appendicitis[92,93].

Diagnosing appendicitis

Acute abdominal pain is a common complaint for which pediatric patients seek emergency medical care[94]. The rate of appendicitis amongst children presenting to the emergency department (ED) with abdominal pain varies between studies. In a retrospective study from Iceland including 1414 ED visits due to acute abdominal pain, 9% of patients were diagnosed with appendicitis[94]. In another study from the USA including 9424 patient visits, appendicitis was reported in 4.3% of the patients[95].

Appendicitis presents a challenge to the medical care givers, since it must be differentiated from other conditions causing similar symptoms. It is of absolute importance to distinguish safely between diagnoses requiring acute attention, among them appendicitis, and benign processes. Despite the development of a range of diagnostic aids, such as clinical prediction scores, laboratory tests and radiology, many children are initially misdiagnosed, especially younger children and girls[32,36]. This is clearly reflected in the rate of negative appendectomies, ranging from 7%[96] to 16.8%[97], but which has been reported to as much as 54% in 0–5-year-old children[98].

Diagnostic aids that can safely differentiate between children with uncomplicated and complicated appendicitis are warranted.

Signs and symptoms

When evaluating a child with acute abdominal pain, it is important to obtain a detailed disease history. Classical anamnestic features of appendicitis include anorexia, fever, nausea, vomiting and migration of pain from the umbilical region to the right lower quadrant. The classical symptoms of a patient with appendicitis are low-grade fever, pain in the right lower quadrant, focal tenderness and guarding. When valued separately, these anamnestic and clinical features have displayed unsatisfying diagnostic values in validating studies. However, when combined, their discriminatory power is increased[99].

Laboratory tests

Routine laboratory tests

C-reactive protein (CRP), white blood cell count (WBC) and absolute neutrophilic count (ANC) are inflammatory markers normally included in the work-up in children with suspected appendicitis. Another diagnostic measurement is the proportion of polymorphonuclear leukocytes (PMN), referring to the percentage of neutrophil

granulocytes, the most abundant of the PMN, of the total WBC count. These blood tests are accessible and relatively cheap, but are non-specific and become elevated in response to inflammation and/or infection, regardless of the underlying cause. Appendicitis is unlikely in patients with one or more normal inflammatory tests[99].

The sensitivity and specificity of CRP have been reported to be 58-93% and 26-82%, respectively[100–102]. The sensitivity and specificity of the WBC count have been reported to be 70-86% and 31-68%, respectively[100–102]. The sensitivity and specificity for ANC have been reported to be 59-97% and 51-90%, respectively[100].

A meta-analysis from 2007 showed a large variance in the diagnostic performance of CRP on pediatric appendicitis, but that a normal CRP in a child with suspected appendicitis reduces the likelihood of appendicitis by half[103]. The same study found that the WBC above age-specific thresholds significantly increased the likelihood ratio of appendicitis by three-fold[103].

Laboratory tests such as these inflammatory markers are not binary and cannot supply a definitive answer as to whether a patient has appendicitis or not; however, the likelihood of appendicitis and complicated appendicitis increase with an increased indication of inflammation, reflected in an elevation of these tests.

Potential novel biomarkers

In a small study, the urine biomarker leucine-rich alpha-2-glycoprotein (LRG) has shown good diagnostic properties in pediatric patients when combined with the PAS: sensitivity 95%, specificity 90%, positive predictive value (PPV) 91% and negative predictive value (NPV) 95%[104]. Incorporating LRG in a novel scoring system including "constant pain, right iliac fossa tenderness and pain on percussion" generated high sensitivity and NPV but low specificity and PPV, indicating a promising non-invasive diagnostic aid to rule out appendicitis in children[105].

Another potential biomarker for pediatric appendicitis is the pro-inflammatory cytokine interleukin (IL)-6. In a meta-analysis including 9 papers the authors found higher levels of IL-6 in the children with appendicitis compared to the controls. However, there was a substantial heterogenicity regarding proposed cut-off values and varying diagnostic performances[106].

Hyponatremia is another promising biomarker for perforated appendicitis in children. In a prospective study of 80 children with appendicitis, plasma sodium concentrations of ≤136 mmol/L had 82% sensitivity and 97% specificity for perforated appendicitis. Sodium concentrations of ≤136 mmol/L were associated with an odds ratio (OR) or 31.9 (95% CI 6.3-161.9) for perforated appendicitis[107]. Another prospective study of plasma sodium concentrations in 184 children with appendicitis displayed a sensitivity

of 95% and a specificity of 89% for perforated appendicitis when the cut-off value of plasma sodium was set to ≤135 mmol/L[108].

Imaging

Ultrasound

Over the last decades, imaging techniques have become an important part of diagnosing appendicitis[109].

Ultrasound (US) is considered the first imaging method of choice for diagnosing appendicitis in children. An inflamed appendix appears as a non-compressible, aperistaltic, thick-walled, blind-ending tubular structure with a maximum diameter of more than 6 mm[110]. The presence of an appendicolith is visualized as a posterior acoustic shadowing[111]. Other features indicative of appendicitis on US are inflammation of the periappendiceal fat, free abdominal fluid, and abscess[112]. A study on 614 children, of whom 28.2% had appendicitis, reported a higher diagnostic accuracy of US on children by dividing the patients into three different categories based on appendix diameter (≤6 mm, >6 mm–8 mm and >8 mm), compared to the traditional binary diameter cut-off. Appendicitis was present in 2.6%, 64.9% and 96.1% of the cases in each group[113].

In a meta-analysis from 2004, the pooled sensitivity and specificity of US for suspected appendicitis in children were 88% and 94%, respectively[114]. In a large singe-center study from 2016 including 3799 US examinations of children with suspected appendicitis, the sensitivity and specificity were 97% and 95%, respectively[115]. The main advantage of US is the lack of radiation exposure. It is, however, user dependent, and often the appendix cannot be visualized due to, for example, abundant subcutaneous tissue, intestinal gas or a retrocecal localization of the appendix[109].

The ability of US to distinguish between uncomplicated and complicated appendicitis in children has also been evaluated. Most of these studies, however, focus on identifying cases of perforated appendicitis, and report varying diagnostic properties[116,117]. Studies stratifying cases of gangrenous appendicitis to the complicated appendicitis group have also reported quite unsatisfying discriminating properties[112,118]. In a small single-center study, the authors found that the sonographic finding "loss of the submucosal layer" yielded a sensitivity of 100% and a specificity of 92% for complicated appendicitis[119].

Computed tomography

Features indicative of appendicitis on computed tomography (CT) include an enlarged lumen with a double-wall thickness of > 6 mm, wall thickening of > 2 mm, absence of intraluminal air, stranding of the periappendiceal fat (edema) and/or the presence of an

abscess or appendicolith. Since the diameter of many appendixes are larger than 6 mm, the importance of secondary signs of appendicitis on CT has been highlighted[120]. CT has a pooled sensitivity and specificity of 94% and 95%, respectively, for diagnosing appendicitis in children[114]. Although this modality displays a higher diagnostic performance, it should be used sparingly in children, considering the risk of cancer associated with exposure to ionizing radiation[121,122]. Significantly reducing the utilization of CT in favor of US does not seem to significantly compromise the diagnostic accuracy[123].

Magnetic resonance imaging

During the last decade, magnetic resonance imaging (MRI) has emerged as a promising alternative imaging technique for diagnosing appendicitis in children. In a meta-analysis from 2017, the pooled sensitivity and specificity for MRI were 98% (95% CI 96-99%) and 97% (95% CI 96-98%), respectively. In this study, the diagnostic accuracy for US, CT and MRI were all high, and did not differ significantly[124]. The main advantage of MRI is the lack of ionizing radiation exposure. The main disadvantage is its lack of availability in many centers.

In summary, imaging for diagnosing appendicitis in children should begin with an abdominal US. If the risk of appendicitis is moderate and the results from the US are equivocal, the medical provider should proceed to observation with or without repeated US, or diagnostic laparoscopy, provided that the suspicion of an appendiceal abscess is low. It has been proposed that one should move forward with CT or MRI in equivocal cases[120], but MRI for suspected appendicitis is seldom used in the clinical setting, and CT is primarily used when an appendiceal abscess is suspected.

A general problem with the imaging diagnostic criteria of the appendix wall thickness is that children with negative appendectomies have had reported diameters of > 6 cm, the commonly used cut-off width for appendicitis[112].

Clinical prediction scores

Clinical prediction scores are statistical tools developed to help clinicians in their decision making. Much like clinicians process information on a patient case in a structured matter, with targeted questions, clinical examinations, and laboratory tests, clinical prediction scores combine multiple predictors to estimate the probability of a certain outcome[125]. The probability estimates generated by clinical prediction scores are more objective and reproducible than the estimates of clinicians. Furthermore, the statistical model can take into consideration far more variables than the human brain[125]. In addition, they are non-invasive, often easy to use and are cost-effective.

Among the most established and well-studied scoring systems for appendicitis are the Alvarado score and the Pediatric Appendicitis Score (PAS). The *Alvarado score,* also called MANTRELS (a mnemonic for the included parameters), was developed in 1986 on retrospectively collected data from 305 hospitalized patients with acute abdominal pain and a mean age of 25 years (range: 4-80 years)[126].

The *PAS* is the first clinical prediction score developed specifically for use on pediatric patients. It was introduced in 2002 and is based on a prospective study of 1170 patients with a mean age of 9.9 years in patients with appendicitis and 11 years in patients without appendicitis (range: 4-15 years)[127].

Both the Alvarado score and the PAS showed very promising results in their primary publications, but on external validations the results have varied with regard to diagnostic accuracy and clinical usefulness[128–130].

In 2008, Swedish surgeons developed the Appendicitis Inflammatory Response *(AIR) score,* based on a prospective study of 545 patients with a mean age of 26 years[131]. Even though the AIR score was developed on adult patients, it outperformed both the Alvarado score and the PAS when validated retrospectively on children[132]. As a result of a large validation study, the low cut-off of AIR score was changed from <5 to <4[133].

In 2018 the pediatric Appendicitis Risk Calculator (*pARC*) was developed from a retrospective study of 2432 children, aged 5-18 years, with suspected appendicitis. It was evaluated in the same study on a sample of 1426 children[134]. This prediction score differs from the others mentioned in that it uses a computerized method that quantifies the risk of appendicitis on a continuous scale (from 0% to 100%), and therefore requires the use of a computer- or internet-based calculator. However, it also uses cut-off levels to assign patients to low, intermediate, and high-risk groups[134]. The pARC outperformed the PAS when validated on 2089 children in 11 emergency departments[135].

The parameters included in the Alvarado score, the PAS, the AIR-score and pARC, as well as their different cut-off levels and maximum scores are presented in Table 1.

Can a negative "speed bump sign" rule out appendicitis?

In the 2012 Christmas issue of the British Medical Journal, Ashdown et al published the study *Pain over speed bumps in diagnosis of acute appendicitis: Diagnostic accuracy study*. They included 101 adult patients that had been surgically assessed due to suspected appendicitis. Sixty-four of the included patients had travelled over speed bumps on their way to the hospital. Out of the 34 with confirmed appendicitis, 33 patients reported increased pain when passing over the speed bumps. This anamnestic factor displayed a quite high diagnostic accuracy with a sensitivity of 97%, a specificity of 30%, a positive predictive value (PPV) of 61% and a negative predictive value (NPV) of 90%[136]. The study was reproduced in 2020 by Eid et al, in a study that displayed similar results[137]. Due to its' low specificity and PPV, the rule-in performance of the "speed bump sign" is quite poor, but perhaps it could be feasible in ruling out appendicitis – at least in adult patients?

The authors of the original article were awarded with the Ig Nobel Prize in 2015.

Table 1. Overview of four clinical prediction scores for appendicitis

Parameters	Alvarado	PAS	AIR	pARC
Age				X
Sex				X
Duration of pain				X
Vomiting			1	
Nausea/vomiting	1	1		
Anorexia	1	1		
Pain in RLQ	2	2	1	
Pain migration to RLQ	1	1		X
Abdominal guarding				X
Maximal tenderness in RLQ				X
Rebound tenderness or muscle defense	1			
Light			1	
Medium			2	
Strong			3	
Hopping/coughing/percussion tenderness in RLQ		2		
Body temperature				
≥ 37.3°C	1			
≥ 38.0°C		1		
≥ 38.5°C			1	
Leukocytosis shift	1	1		
WBC count				
> 10 x 10^9/L	2	1		
10-14 x 10^9/L			1	
≥ 15 x 10^9/L			2	
PMN				
70-84%			1	X
≥ 85%			2	
CRP				
10-49 mg/L			1	
≥ 50 mg/L			2	
Total score	**10**	**10**	**12**	**100%**
Low risk	0–4	0–5	0–3/4*	0-14%
Intermediate risk	5–6		4/5–8*	15-84%
High risk	7–10	6–10	9–12	85-100%

PAS: Pediatric Appendicitis Score; AIR: Appendicitis Inflammatory Response score; pARC: pediatric Appendicitis Risk Calculator; RLQ: Right Lower Quadrant; WBC: White blood cell; PMN: Polymorphonuclear leukocytes; CRP: C-reactive protein. *The original cut-off point for AIR score of <4[131] was revised to <5 in a validating study[133].

Treatment of appendicitis

For over a century, appendicitis has usually been treated through surgical removal of the appendix – an appendectomy. While the incidence of appendicitis in Western countries has stabilized during the 21st century, the incidence of appendectomy has decreased steadily[33]. The reasons for this are probably multifactorial with advances in both the diagnostic arena as well as the surgical one. For example, a new regimen of conservative treatment of uncomplicated appendicitis with antibiotics without surgery is gaining more and more popularity.

Surgical treatment

For more than 100 years, open appendectomy (OA) was the standard treatment for appendicitis. Since the introduction of laparoscopic appendectomy (LA) many studies have compared the surgical methods.

In a meta-analysis from 2010 including 25 randomized controlled trials (RCTs) comparing OA and LA in a total of 4694 patients, LA resulted in less postoperative pain, faster postoperative rehabilitation, shorter hospital stays and fewer postoperative complications. LA, however, yielded a longer operative time[138]. Another meta-analysis of 33 RCTs including both adult and pediatric patients confirmed these results in adults but did not find any significant differences between OA and LA in children[139].

In a meta-analysis from 2006 of 23 studies comparing OA and LA in children, the authors concluded the rates of postoperative complications were lower for LA, and that the operation time for LA was not significantly longer compared to that of OA. Postoperative hospital stay was shorter for LA[140]. In another meta-analysis from 2012, including 26 studies of a total of 123,628 children, advantages of LA over OA in children in terms of wound infections, postoperative ileus, shorter hospital stays, and a more rapid recovery were reported. Operating time for complicated appendicitis was significantly shorter for OA, but the operating times for OA versus LA were comparable for simple cases. The authors strongly recommended LA over OA[141]. Another advantage of LA is that if a healthy appendix is identified, the entire abdominal cavity can be examined for other causes of abdominal pain.

The results of a Swedish retrospective cohort study from 2016 including 1745 children supported the longer operating time for LA, but did not find any significant differences in complication rates between OA and LA. The authors concluded that the shorter hospital stay was attributed to a general trend towards shorter stays, regardless of the surgical method[142]. A nationwide Swedish study including 38,939 children found that

LA was associated with fewer surgical site infections and a lower risk of postoperative small bowel obstruction compared to OA, unless conversion to OA was needed[143].

Since the introduction of laparoscopic surgical access, the minimally invasive techniques for appendectomy have continued to develop. Laparoscopic appendectomy is usually performed with a three-port access, but techniques using a two-port[144] as well as a single-incision multi-port access[145] have been described.

Timing of surgery

The results from an American retrospective study including 2429 children < 18 years of age indicated that an in-hospital delay of appendectomy of up to 24 hours does not affect the risk of developing perforated appendicitis or postoperative complications[146]. A Swedish retrospective study including 2756 children < 15 years of age did not find any associations between increased in-hospital delay of up to 36 hours or increased risk of developing perforated appendicitis and adverse outcomes after appendectomy[147]. The American Pediatric Surgical Association recommends that appendectomy is performed within 24 hours of presentation[148].

In contrast to previous views of appendicitis as an emergency condition, it is now evident that in children presenting after hours it is safe to postpone appendectomies to the following day in clinically stable patients with no obvious signs of perforation.

Nonoperative management

Both OA and LA are considered low-risk procedures. However, all surgical procedures are encumbered with risks and complications associated with both general anesthesia and the surgical procedure itself. The advantage of a nonoperative management (NOM) is therefore the avoidance of the short- and long-term risks associated with surgery. This must be countered with the risk of conservative treatment, namely the risk of perforation and recurrent appendicitis.

In a meta-analysis from 2021 including 21 studies either comparing NOM to appendectomy or reporting outcome of NOM of appendicitis in children, the acute symptoms of nonperforated appendicitis resolved without appendectomy in 92% of the patients. Of the patients, 16% underwent appendectomy after their initial hospital stay, either due to recurrent appendicitis or recurrent abdominal pain with normal appendectomy. The 21 studies included in the meta-analysis differed in study design and only one was an RCT. The authors concluded that nonoperative treatment for nonperforated appendicitis in children is safe and efficient, but they underlined the

need for larger RCTs[149]. The presence of an appendicolith is associated with higher failure rates of NOM[150,151] and excluding patients with an appendicolith improves the treatment efficacy of NOM[152].

To date, NOM most often implicates treatment with antibiotics. However, only supportive care with intravenous fluids, analgesia and antipyretics seems to be as effective as antibiotic treatment in adult patients with CT-verified uncomplicated appendicitis[153]. This further suggests that many cases of uncomplicated appendicitis have the potential to resolve spontaneously, without the necessity of antibiotic treatment (or operation).

Different outcomes

Appendicitis is associated with morbidity and, although very unusual, mortality. Among the most common complications following appendicitis and appendectomy are surgical site infections, postoperative abscesses, and small bowel obstructions.

Surgical site infections have been reported to occur in overall 2.8%-5.1%[143,154,155] of children who have undergone appendectomy. Infections are more common after complicated appendicitis, and after open appendectomy[143,155].

Postoperative intra-abdominal abscesses occur in 7.4%-13.2% of children with perforated appendicitis[156–158].

The overall risk of adhesive small bowel obstruction after appendectomy is low, between 0.2% and 1.9% in Swedish children[143,159]. It is significantly related to perforated appendicitis and postoperative intra-abdominal abscesses and is more common after OA compared to LA[141,157,158].

The mortality rate of appendicitis in high-income countries is very low. In a national Swedish study, only 2 out of nearly 39,000 children died within 30 days of their appendicitis episode[143].

A brief introduction to immunology

The immune system is designed to prevent and eradicate infections. It is dependent on its important ability to differentiate pathogens from host cells and consists of two major lines of defense: innate and adaptive immunity (Figure 4).

The innate (or native/natural) immunity mediates the first line of defense against pathogens through mechanisms always present in a healthy individual. Epithelial and chemical barriers prevent infections, while phagocytic cells (neutrophils and macrophages), natural killer (NK) cells and plasma proteins (such as those involved in the complement system[160]) eliminate the pathogen. The innate immunity also communicates with the adaptive immune system and instructs it to react effectively against the pathogens. In turn, the adaptive immune system uses mechanisms of the innate immunity to neutralize pathogens.

Although the innate immunity is quite effective, many pathogenic microorganisms can break through this first line of defense and need to be fought by the adaptive (or specific/acquired) immunity. The immunological mechanisms of the adaptive immune system are broader and more refined than the rapid processes of the innate immunity, but since it adjusts to the presence of microorganisms, it has a slower onset compared to the innate immunity[161]. Adaptive immunity consists of humoral and cell-mediated immunity. Humoral immunity is mediated through antibodies, which are produced by B cells and secreted into the bloodstream and mucosal fluids. Here they recognize and help neutralize and eliminate microbes. However, antibodies cannot access microbes that live and divide inside infected cells. Instead, the cell-mediated immunity, mediated by T lymphocytes, defends the body against such infections. The T lymphocytes recognize antigens produced by intracellular microbes, and either kill infected host cells themselves or activate phagocytes[162].

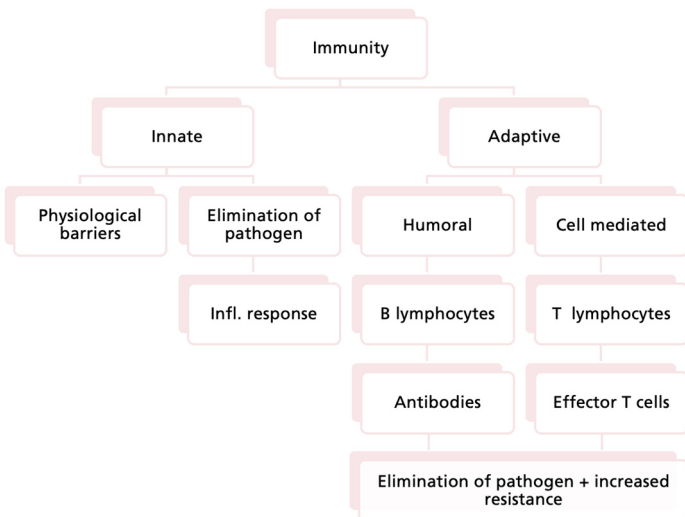


Figure 4. A simplified overview of the innate immunity and the adaptive immune systems.

Some important cells of the immune system

Leucocytes, or WBC, include all cells of the immune system. Among them are the myeloid cells, which engulf and destroy pathogens. The myeloid cells include mononuclear and polymorphonuclear phagocytes. The mononuclear phagocytes include the *macrophages*, which are almost the tissue-bound equivalent of the circulating *monocytes*. Not only are the macrophages important for their phagocytic properties, but they also function as *antigen presenting cells* (APCs), meaning that they present pathogen antigens to T cells and thereby help initiate the adaptive immune responses[163].

Neutrophils, basophils, and eosinophils make up another important groups of leukocytes – the polymorphonuclear phagocytes or granulocytes. The *neutrophils* in particular are important key players during acute inflammation. They are the most abundant leukocyte in the blood circulation, have a short life span of around 6 hours, and their numbers may be elevated greatly as a response to severe infection. In addition to killing pathogens through phagocytosis, they can release antibacterial proteins from their granules into the extracellular space[164]. Since appendicitis is histopathologically defined as neutrophil infiltration into the lamina muscularis[52], it is safe to say that these cells play an important role in the condition.

Eosinophils contain acidophilic cytoplasmic granules that have a high affinity for the red dye eosin, and other acidic dyes[165]. Along with mast cells and basophils, the eosinophils are key regulators of the immunological mechanisms associated with allergy and asthma[166]. They are also important in the defense against parasites, such as helminths[167].

Mast cells reside in all connective tissues and are primarily involved in the inflammatory mechanisms that cause allergic symptoms. They express the high-affinity IgE-receptor (FcεRI) and, when activated by the presentation of a the IgE-specific allergen, they release immune mediators of which histamine is the most abundant. They also secrete Th2 cytokines[166]. *Basophils* share many features with the mast cells, but they are primarily circulating cells[166].

Lymphocytes are present in blood, lymphoid tissue and practically all organs and include B and T cells as well as NK cells. The *T cells* undergo maturation and selection processes in the thymus and are key players in the cell-mediated immune system[168]. *B cells* arise from hematopoietic stem cells in the bone marrow and are through their production of antibodies key players in the humoral immune system[168]. *NK cells* are important for the innate immunity through recognition and killing of pathogens. They also regulate the adaptive immune responses through secretion of cytokines[169].

T helper cells

T helper (Th) cells can be viewed as the conductors of the large orchestra that is the adaptive immune system. To help the immune system work in a coordinated and goal-oriented matter, the Th cells use cytokines, small proteins by which the immune system communicates, as their conductor's baton. As conductors, they have a significant role during, for example, infection, and autoimmune and inflammatory diseases[170].

During the last three decades, studies have revealed a great heterogenicity among the Th cells regarding their different cytokine expressions. They have, based on their functions, been categorized into subsets, for example Th1, Th2, and Th17. All Th cells stem from naïve T cells and differentiate into one of the different subsets of Th cells after activation through specifying cytokines[171].

Th1 cells are associated with the cellular immune system and are important for example in the defense against intracellular bacteria by signaling to macrophages to kill pathogens located in the phagosomes (the vesicle containing a pathogen that has been ingested by a phagocyte) and by activating cytotoxic T cells to kill infected cells. The Th1 cells' signature cytokines are interferon-γ (IFN-γ), interleukin (IL)-2 and tumor necrosis factor (TNF)-α[171].

Th2 cells are associated with the humoral immune system and are important in the defense against extracellular pathogens, such as helminths, and their signature cytokines are IL-4, IL-5, IL-9, and IL-13[172]. They are also associated with the cellular responses seen in asthma and allergic reactions[173], and with pregnancy to promote fetal tolerance[174].

Th17 cells are primarily found in the gastrointestinal tract where they help regulate the gut microbiota, but they are also important in the defense against fungal as well as intra- and extracellular bacterial infections[171]. Their signature cytokines are IL-17A, IL-17F and IL-22[171].

Immediate (Type I) hypersensitivity – also known as allergy

Hypersensitivity reactions are abnormal or exaggerated immune responses that are potentially harmful to the human body – the precise thing that the immune system aims to protect. These occur when the immune system either responds to self-antigens (autoimmunity), or when responses to foreign antigens are dysregulated or uncontrolled. Hypersensitivity reactions are categorized according to the immunological mechanisms involved. The most common disorder of the immune

system is immediate (or type I) hypersensitivity, also known as allergy (Figure 5). Key players in these responses are Th2 cells, IgE antibodies, eosinophils, and mast cells[162,166].

In allergic individuals, the first encounter with certain allergens (antigens that trigger allergic reactions, for example pollen or food) leads to activation of Th2 cells and secretion of IL-4 and IL-13 to stimulate B lymphocytes specific to that allergen to become IgE-producing plasma cells[162,175]. In allergic individuals these plasma cells produce large amounts of IgE antibodies in response to antigens that do not induce IgE production in non-allergic individuals. This caters for the diagnosis of allergy to certain allergens by measuring the concentrations of their specific antibodies in serum. The IgE antibodies in turn bind to high affinity receptors (FcεRI) on mast cells, a process called sensitization, leaving them coated with IgE antibodies specific to the allergen to which the individual is allergic. One can compare these coated mast cells to a minefield – the allergic individual is now only a presentation of a harmless antigen away from a potentially massive immunological chain reaction.

When the individual is re-exposed to the specific allergen, the allergen must be presented to at least two FcεRI-receptors on the mast cell to elicit the responses in the mast cell responsible for the allergic symptoms. When activated, the mast cell almost immediately releases its granules (a process called degranulation) containing vasoactive aminases, primarily histamine, and proteases. Histamine increases the blood flow through dilatation of small blood vessels and increased vascular permeability and causes a transient smooth muscle contraction. The proteases may cause tissue damage. In addition to degranulation, the mast cells synthesize and secrete cytokines which recruit eosinophils, neutrophils and Th2 cells causing an inflammation through release of proteases and additional cytokines exacerbating the inflammatory reaction. IL-5 secreted by Th2 cells and mast cells is responsible for activation of eosinophils. Th2 cells also secrete IL-13, which stimulates the airway epithelium to secrete mucus[162].

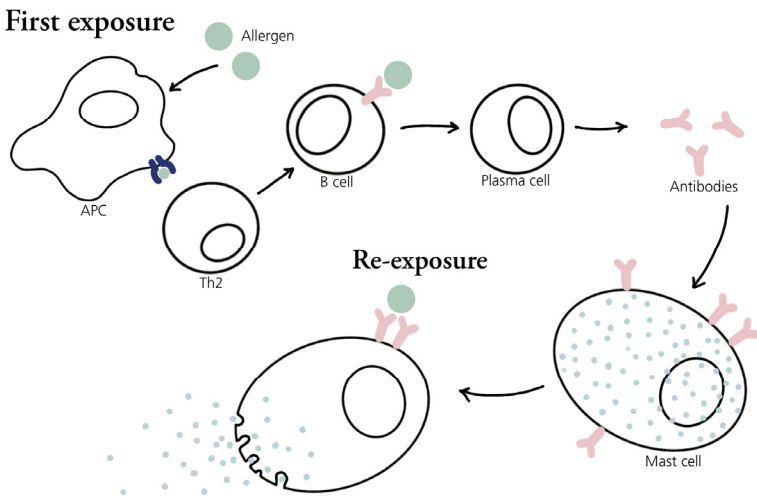


Figure 5. An overview of the series of events involved in an allergic (type 1 hypersensitivity) reaction.

Cortisol, the HPA axis, and stress

Cortisol is the main glucocorticoid hormone in humans. It is produced in the zona fasciculata (the middle zone) of the adrenal cortex, in levels regulated by the hypothalamus-pituitary-adrenal (HPA) axis. The HPA axis is an elegant network of interactions between the hypothalamus, the pituitary gland, and the adrenal glands. The hypothalamus releases corticotropin-releasing hormone (CRH) in amounts influenced by stress, illness, circadian rhythm, and blood levels of cortisol. CRH, in turn, stimulates the anterior lobe of the pituitary gland to secrete adrenocorticotropic hormone (ACTH), which stimulates the adrenal cortex to release cortisol into the blood stream. Included in the HPA-axis is a negative feedback system, in which the cortisol acts back on the hypothalamus and the pituitary to suppress the production of CRH and ACTH[176].

Cortisol has vital homeostatic functions, for example the maintenance of adequate blood pressure and glucose levels. When humans are subjected to stress, either physical or psychological, the activity of the HPA axis is increased to protect us by maintaining homeostasis. However, the same mechanisms that help us to survive acute stressors can be upregulated for prolonged periods of time as a response to chronic stress, causing adverse events in our brain and body. It is therefore important to distinguish between the protective effects of an adequate hormone-mediated response to short-term stress (allostasis) from the potential harmful effects, the "wear and tear on the body", caused by long-term stress and dysregulation of stress mediators (allostatic load)[177].

Stress, both acute and chronic, results in alterations of the immune system[178], and cortisol mediates important immunosuppressive mechanisms to prevent the immune system from overshooting. For example, it has been shown that cortisol affects the balance of the different Th cell subsets, primarily by strongly suppressing the Th1-mediated immune responses through inhibition of TNF-α, IL-12 and IFN-γ[179,180]. Cortisol also suppresses the activity of the Th2-mediated immune responses[180,] but to a much lesser extent, creating a shift towards the Th2-dependent immune responses[179,180.]

Measuring cortisol offers the possibility of evaluating the activity of the HPA axis, and thereby the levels of stress. Cortisol is usually measured in blood, salivary or urine samples, but these single measurements are highly influenced by the circadian rhythm and stress-related fluctuations of activity of the HPA axis. Hence, an assessment of the long-term activity of the HPA axis using these analysis methods warrants repeated sampling over different times of the day and for several days[181]. Instead, the measurement of cortisol concentrations in hair (hair cortisol concentrations, HCC) has emerged as an increasingly popular method of evaluating the long-term activity in the HPA axis[181]. When hair grows from the hair follicles, cortisol molecules are incorporated into the hair strands in concentrations reflecting the activity of the HPA axis during the time of growth, leaving historical imprints, much like age rings in trees[181]. Since the hair on the back of the scalp grows at a rate of approximately 1 cm/month, hair can be cut close to the scalp, segmented, and analyzed with measurements of HCC, retrospectively reflecting the cortisol secretion during the last months[182].

The exact mechanisms of cortisol incorporation into hair are not completely understood. Multiple sources have been proposed, including passive diffusion from capillaries, sweat and sebum (Figure 6)[181.]

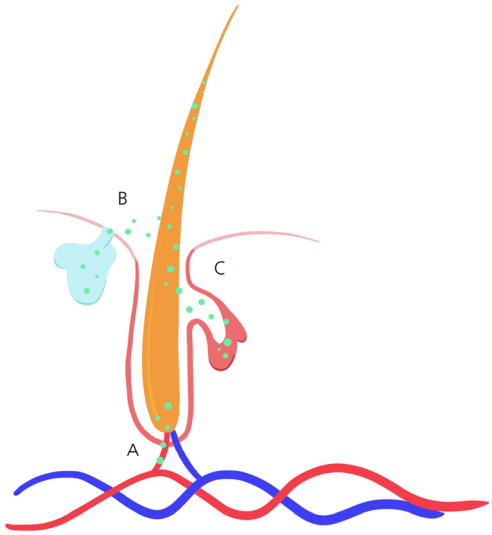


Figure 6. Cortisol is proposed to be incorporated into hair from the blood stream (A), sweat glands (B) and sebaceous glands (C).

"Felix, qui potest rerum cognoscere causas"

"Fortunate is he, who is able to know the causes of things"

Virgil,
verse 490 of Book 2 of Georgics (29 BC)

Knowledge gaps and research questions

Due to the heterogenicity in the natural course of appendicitis, the condition is now usually classified as uncomplicated (with the potential for spontaneous resolution) or complicated (with an inflammation progressing to gangrene and perforation). Both epidemiological and clinical studies indicate that an individual's immune responses are involved in propelling the inflammation towards an uncomplicated or a complicated disease course. Complicated appendicitis seems to be associated with a Th1/Th17-dependent response, while uncomplicated appendicitis seems to be associated with a Th2-dependent immune response. However, the etiology and pathogenesis of appendicitis are still not completely understood, including the potential influence of different inflammatory processes.

Which immunological mechanisms are part of the etiology and pathogenesis of appendicitis in children? How do the mechanisms differ between uncomplicated and complicated disease?

Clinically diagnosing appendicitis in the pediatric population is still hampered by difficulties. This results in high rates of initial misdiagnosis which, in turn, has two major negative consequences: negative appendectomies and delayed diagnosis, which increase both patient morbidity and health care costs.

How can a clinician predict the risk of appendicitis in pediatric patients with higher accuracy?

Today there are no diagnostic aids that can accurately distinguish uncomplicated from non-perforated complicated appendicitis. Based on the previously proposed association of different immune responses in uncomplicated and complicated appendicitis, mediators involved in these immunological processes could possibly be used clinically to help distinguish uncomplicated from complicated appendicitis. This would also facilitate the selection of patients in future research on novel treatment options, such as non-operative management with or without antibiotics.

Are there any novel biomarkers that can distinguish between uncomplicated and complicated appendicitis in children?

Aims

The overall aims of this thesis were to increase the knowledge of how the inflammatory processes anteceding an appendicitis episode can be categorized, modulated, and detected.

The specific intentions for the conducted studies were:

Paper I

To prospectively evaluate the diagnostic accuracy of four clinical prediction scores for children with suspected appendicitis.

Paper II

To evaluate the risk of complicated appendicitis in children with IgE-mediated allergy.

Paper III

To compare the relative and absolute risk of complicated appendicitis in allergic and non-allergic children, and to investigate if the protective effect of allergy was modified by seasonal antigen exposure, allergy medication and the temporal relationship between allergy diagnosis and the appendicitis episode.

Paper IV

To investigate the associations of stress, measured as HCC, with appendicitis and complicated appendicitis in children.

Paper V

To evaluate the associations of IgE and Th2-associated interleukins with complicated appendicitis in children.

Methods

Clinical setting

The patients in Papers I, II, IV and V were assessed and/or treated at the tertiary center for pediatric surgery, Skåne University Hospital, Lund, Sweden. The center has an uptake area of approximately 350,000 inhabitants with primary surgical care for children under 15 years of age and of 1.3 million inhabitants for surgical care for children under 3 years of age. Children with suspected appendicitis are referred for pediatric surgery consultation from either a general practitioner or from a pediatrician at the pediatric ED. All studies included children aged 15 years or younger, hereafter referred to as children or patients.

Definitions

Routine management of appendicitis

All children included in the clinical studies were treated at the department of Pediatric Surgery at Skåne University Hospital, Lund, Sweden. Participation in any of the studies did not alter the management of suspected or confirmed appendicitis.

Children with suspected appendicitis were referred to a resident or consultant pediatric surgeon by either a pediatrician, general practitioner or by a triaging nurse. Diagnosis was generally made based on disease history, clinical examination, routine laboratory blood tests (CRP, WBC and ANC). Sometimes the children underwent US or, rarely, CT to confirm or exclude the diagnosis.

Appendicitis was always treated with appendectomy and never conservatively with antibiotics. The only exceptions were some cases with appendicular abscesses. Appendectomy was performed either laparoscopically with two or three ports or through a grid iron incision in the right lower quadrant. All children received prophylactic perioperative antibiotics with trimethoprim/sulfamethoxazole and

metronidazole in doses based on patient age and weight, respectively. Children with gangrenous or perforated appendicitis were treated with postoperative antibiotics initially administered intravenously, and then orally for an additional couple of days, based on their clinical and laboratory status.

Uncomplicated and complicated appendicitis

Uncomplicated appendicitis was defined as phlegmonous appendicitis. Complicated appendicitis was defined as either gangrenous appendicitis, perforated appendicitis, or appendicular abscess.

The diagnosis was based on the histopathological criteria of infiltration of neutrophil granulocytes in the muscularis propria layer. Gangrenous appendicitis was defined as transmural inflammation on histology and necrosis seen macroscopically as a black or gray discoloration, without any of the criteria for perforation. Perforated appendicitis was defined as a visible hole in the appendix and/or presence of pus, intestinal content or an appendicolith in the abdominal cavity. Appendicular abscess was defined as an inflamed appendix and an abscess adjacent to the appendix and was diagnosed either by imaging studies (US, CT, or MRI) or perioperatively.

IgE-mediated allergy

In Paper II, information on allergy status was collected from the regionwide medical records, including surgical and anesthesia records. In Paper III, the definition of allergy was based on a previously established Swedish algorithm for allergy diagnosis, based on ICD (International Classification of Diseases)-10 codes from the children's medical records, and/or ATC (Anatomical Therapeutic Chemical) codes of dispensed prescribes allergy medications[183].

Study designs and study populations

Paper I

The design was a prospective institution-based cohort study including all children with symptoms indicative of appendicitis referred to the on-call pediatric surgeon (resident or consultant) for evaluation at the pediatric ED during March 1st 2016 to February 28th 2018 (Table 2). Exclusion criteria were a previous episode of suspected

appendicitis, severe chronic illness, or ongoing treatment with anti-inflammatory drugs. Data regarding medical history, findings on clinical examination and laboratory were recorded in a study protocol. From this, the patients' scores for four different clinical prediction scores (PAS, AIR score, Alvarado score and pARC) were later derived. Medical records were reviewed to obtain the patients' final diagnoses and results from histopathological examinations. Primary outcomes were appendicitis and complicated appendicitis. Secondary outcomes were negative appendectomies and missed cases of appendicitis.

A total of 345 children were eligible for inclusion. There were 17 patients who were excluded due to missed inclusion or possessing any of the exclusion criteria, and another 10 children were excluded due to missing data. Hence a total of 318 children remained for analyses. There were 151 (47%) children with appendicitis and 67 (44%) of these had complicated appendicitis. The median age in the appendicitis group was 11 (min-max 2-14) years and 102 (68%) were boys. In the non-appendicitis group, the median age was 9 (min-max: 2-14) years, and 74 (44%) were boys. Among the children with appendicitis, 142 underwent appendectomy. There were eight negative appendectomies in the no appendicitis-group.

Paper II

The design was a retrospective institution-based cohort study including all children who had undergone appendectomy during January 1st 2007 and July 31st 2017 (Table 2). Exclusion criteria were normal findings on histopathological examination (negative appendectomy). The following information was gathered from medical records: age, sex, symptom duration, presence of an appendicolith, length of in-hospital stays, number of primary care visits during the last 12 months before appendectomy, allergy status and allergens, seasonal antigen exposure (in which month the appendectomy was performed) and use of allergy (antihistamine) medication. Primary outcome was complicated or uncomplicated appendicitis. Secondary outcomes were length of in-hospital stay after surgery, and reoperation rate (any abdominal surgery requiring general anesthesia within 30 days after appendectomy). Primary exposure was occurrence of any IgE-mediated allergy. Potential confounders were age, sex, symptom duration, presence of appendicolith, number of primary care visits during the last 12 months before surgery, seasonal antigen exposure, and ongoing use of allergy medication.

A total of 689 children were initially eligible for inclusion. After exclusion of 82 children with negative appendectomies and two with missing data regarding allergy status, 605 children remained. In the total cohort, the median age was 10 (IQR 7-12)

years and 381 (63%) were boys. There were 102 (16.9%) children with IgE-mediated allergy. The median age in the allergy group was 11 (IQR:9-13) years, and in the no allergy group 10 (IQR: 7-12) years. In the allergy group 66 (64.7%) were boys, compared to 315 (62.6%) in the no allergy group.

Paper III

The design was a nationwide cohort study comparing allergic and non-allergic children for absolute and relative risk of complicated appendicitis (Table 2). All children born between 2000 and 2010 were included and monitored in national registers for occurrence of allergy and appendicitis, from birth until December 31, 2014. The primary outcome was uncomplicated and complicated appendicitis. The study consisted of two parts. First, a cross-sectional analysis was performed where only the children with appendicitis were analyzed and compared on the basis on allergy status for frequencies and OR of complicated appendicitis. Adjustments were made for age, sex, and parental education level. Effect modifications of the risk of complicated appendicitis were evaluated for seasonal antigen exposure, allergy medication and timing of allergy onset. In the second part of the study, a longitudinal analysis was performed, in which each allergic child was matched with three never-allergic controls based on age, sex and year of birth. These cohorts were followed and analyzed for differences in absolute risk and hazard ratios of complicated and uncomplicated appendicitis. Children who died or migrated during the inclusion time were excluded. Children were censored at primary outcome, migration, death, end of study or colectomy for non-appendicitis reasons.

A total of 1,122,571 children were included. Of these 227,128 (20%) developed IgE-mediated allergy and 6367 (0.6%) developed appendicitis. In the cross-sectional analysis including 6367 children with appendicitis, 1351 (21%) had allergies. The median age among the allergic children was 9 (IQR 7-11) years, and among the non-allergic children 8 (IQR 5-19) years. In the allergy group 849 (63%) were boys, compared to 2809 (56%) in the no allergy group.

Paper IV

The design was a prospective institution-based case-control study in which patients were enrolled at the inpatient pediatric surgery ward after appendectomy or during conservative treatment for an appendicular abscess. The inclusion period stretched from 2017 to 2019 (Table 2). Healthy controls were recruited through personal contacts, from hospital staff and by advertisements at local food markets and a family

gym. Exclusion criteria were a previous episode of appendicitis, severe chronic illness or ongoing immune modulating therapy at inclusion, a hair sample (the hairs covering the area of approximately 0.5 cm^2) was cut from the posterior vertex area. Additionally, a questionnaire, covering the number of viral or bacterial infections, surgeries, and serious life events, was filled out with help from one of the researchers. Serious life events were defined as, for example, the passing of a close relative, parental divorce, or domestic abuse. Medical records were reviewed to obtain results from histopathological examinations.

A total of 53 patients and 90 healthy controls were included. After exclusion due to missing data, ongoing medication with inhalation corticosteroids, HCC out of standard curve and too short hair samples, 51 patients and 82 controls remained for further analyses. Another 11 patients and 16 controls were excluded from analyses of HCC 4-6 months prior to sampling due to hair shorter than 6 cm. The patients' median age was 9 (min-max 1-13) years, and 26 (51%) were boys. 34 (67%) had complicated appendicitis. The controls' median age was 6 (min-max 1-15) years and 38 (41%) were boys.

Paper V

The design was a prospective institution-based cohort study including patients referred to the on-call pediatric surgeon for evaluation of symptoms indicative of appendicitis at the pediatric ED during December 9th 2017 to February 16th 2021 (Table 2). Exclusion criteria were previous episode of suspected appendicitis, severe chronic illness, or ongoing treatment with anti-inflammatory drugs. Data regarding medical history, symptom duration and allergy status were registered. Study blood samples were only collected if other blood tests were indicated clinically. Medical records were reviewed to determine the patients' final diagnoses and the results from histopathological examinations, as well as occurrence of an appendicolith. The primary outcome was complicated appendicitis.

A total of 215 children were eligible for inclusion, and after exclusion due to any of the exclusion criteria or missing data, a total of 178 children remained for further analyses. Of these 138 (78%) had appendicitis, out of which 58 (42%) were complicated cases. The median age was 10 (IQR 8-12) years and 103 (58%) were boys.

Table 2. Overview of the study subjects in Papers I-V

	Study period	Eligible cohort	Excluded	Study subjects (n)	Age and sex
I	March 2016 – Feb 2018	Children with suspected appendicitis	10 missed 5 declined participation 1 previous appendicitis 1 cortisone medication 10 missing data	151 children with appendicitis - 67 complicated 167 children with other diagnoses	Appendicitis: 11 (2-14)[a] years 68% boys Other diagnoses: 9 (2-14)[a] years 44% boys
II	Jan 2007 – Jul 2017	Children with an intraoperative diagnosis of appendicitis	82 negative appendectomy 2 without allergy data	605 children with appendicitis - 102 allergic - 503 non-allergic	Allergy: 11 (9-13)[b] years 65% boys No allergy: 10 (7-12)[b] years 63% boys
III	Jan 2000 – Dec 2014	All children born in Sweden between 2000 and 2010	Children who died or migrated between 2020 and 2010	1,112,571 children - 227,128 allergic - 6,367 appendicitis	In the cross-sectional analysis: Allergy (n=1351): 9 (7-11)[b] years 63% boys No allergy: 8 (5-10)[b] years 56% boys
IV	2017 – 2019	Children with appendicitis and healthy controls	Appendicitis: 1 ICS medication 1 HCC out of standard curve Controls: 3 missing data 1 ICS medication 4 hair < 3 cm	51 children with appendicitis - 34 complicated 86 healthy controls	Appendicitis: 9 (1-13)[a] years 51% boys Controls: 6 (1-15)[a] years 41% boys
V	Dec 2017 – Feb 2021	Children with suspected appendicitis	X (unknown number) missed 4 declined participation 5 without blood sampling 2 previous symptoms 1 ongoing methotrexate treatment 1 ongoing allergen immunotherapy 21 missing data	138 children with appendicitis - 58 complicated 40 children with other diagnoses	Appendicitis: 10 (8-12)[b] years 63% boys Other diagnoses: 11 (9-12.75)[b] years 40% boys

[a]Age presented as median (min-max); [b]Age presented as median (IQR); ICS: Inhalation Corticosteroid; HCC: Hair Cortisol Concentration.

Laboratory methods

Reference intervals of standard blood runs

Routine laboratory blood tests were analyzed at the department of Clinical Chemistry, Skåne University Hospital, Lund, Sweden, according to standard protocol. Reference intervals vary with age (Table 3).

Table 3. Reference intervals of stantard laboratory tests according to age

Laboratory test and age intervals	Reference interval
CRP	< 3 mg/L
WBC	
3 months – 3 years	6 – 16 x 10^9 /L
3 – 6 years	5 – 15 x 10^9 /L
7 – 15 years	7 – 15 x 10^9 /L
ANC	
1 – 5 years	1.6 – 6.5 x 10^9 /L
5 – 10 years	2.4 – 6.5 x 10^9 /L
10 – 15 years	1.2 – 7 x 10^9 /L

CRP: C-reactive protein; WBC: white bloodcell count; ANC: absolute neutrophil count.

Analysis of hair cortisol concentrations

The hair samples were collected in the following manner: A small amount of hair covering approximately 0.5 cm^2 of the posterior vertex was held firmly between two fingers and cut as close to the scalp as possible. This area of the scalp is preferred for hair sampling due to its uniform growth rate[181,184]. The newly cut edges were fixed together using aluminium foil and the hair was stored in plastic test tubes in room temperature for up to 24 months before analyses. The hair strands were sectioned into two parts, each 3 cm long, measured from the ends closest to the scalp. These sections reflected the HCC during the 0-3 and 4-6 months prior to sampling since hair grows at a rate of approximately 1 cm per month (Figure 7).

HCC were analyzed at the Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden.

The hair samples were weighed and put in new test tubes along with a 5 mm steel ball. They were then frozen in liquid nitrogen for 2 minutes and minced at 30 Hz for 20 seconds, leaving a fine hair powder. 1 mL of ethanol was added to each test tube, and the test tubes were put on a horizontal stirrer at room temperature for >10 hours. After

this, they were centrifuged and 700 µL of the supernatant was moved to another sample tube for lyophilization.

The lyophilized hair sample extracts were dissolved in 150 µL phosphate buffer containing bovine serum albumin and triton-X. Cortisol levels were assessed through radioimmunoassay, using a gamma counter. Hair samples of 3-10 mg were needed to maintain a repeatability coefficient of variation below 8% for the combination of hair extraction and measurement of cortisol by the radioimmunoassay.

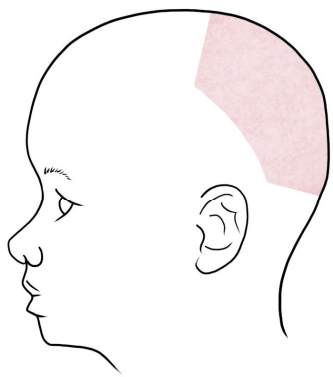


Figure 7. The posterior vertex area, where the hairs grow at a the most uniform rate of approximately 1 cm per month.

Analysis of IgE and interleukins

Blood samples were collected at the pediatric ED and sent to the department for Clinical Chemistry, where the test tubes were left standing upright for 30 minutes before centrifugation at 2000G. Serum was then allocated to between one and three separate test tubes containing 0.5 mL, depending on the amount of serum available, and frozen to minus 80 degrees Celsius. The frozen serum samples were stored at the regional biobank until analyzed.

Human serum IgE was analyzed using a sandwich enzyme-linked immunosorbent assay (ELISA) kit (ab195216, Abcam, The Netherlands) according to the manufacturer's manual. Standard (0, 0.11, 0.18, 0.26, 0.40, 0.59, 0.89 and 1.33 ng/mL) serum samples diluted 1:800 (50 µL/well in duplicate) and antibody cocktail (50 µL/well) were added. After 1 hour's incubation at room temperature on a shaker, the plates were washed and tetramethylbenzidine (TMB) substrate 100 µL/well was added. The

reaction was stopped after a 10-minute incubation in darkness and the absorbance was measured at 450 nm. Intra-and inter assay coefficients of variations (CV) were 4.0% (n = 4) and 3.5% (n = 3), respectively, and average recovery was 108% (range 98.4-118%).

The age specific reference intervals according to the Department of Clinical Immunology and Transfusion medicine, Region Skåne, are presented in Table 4.

Table 4. Reference intervals of total serum IgE according to age

Age (years)	**Reference interval** (ng/mL)
> 1	< 31.2
> 2	< 55.2
> 3	< 76.8
> **4**	< 96
> 5	< 115.2
> 6	< 134.4
> 7	< 151.2
> 8	< 170.4
> 9	< 187.2
> 10	< 204

The Mesoscale Discovery® (MSD, Maryland, USA) U-PLEX® multiplex assay biomarker group (K15067L-2, MSD) was used to perform the selected analyses of IL-4, IL-9 and IL-13 in serum by electro-chemiluminescence detection. Biotinylated IL-4, IL-9 and IL-13 capture antibodies, 50 µL/well, were added to the U-PLEX™ multiplex SECTOR® plate and incubated overnight at 4°C on a shaker. Calibrators 50 µL/well and serum (diluted 1:2) 50 µL/well were added after the plates had been washed three times with MSD wash buffer. A 1-hour incubation at room temperature was followed by a new washing procedure and a SULFO-TAG™ detection antibody, 50 µL/well, was added. After a second 1-hour incubation and a washing procedure, 150 µL MSD GOLD™ read buffer in each well was added and the plates were read on an MSD instrument. The intensity of emitted light is proportional to the amount of IL-4, IL-9 and IL-13 in the wells.

Statistical analyses

Table 5. A summary of the statistical tests used in this thesis

Application	Test
Assessing differences in categorical or binary variables	
Two groups	Chi-squared test Fisher's exact test
Assessing differences in continuous variables	
Two groups	Student's t-test (parametric) Mann-Whitney U-test (non-parametric)
More than two groups	Kruskal-Wallis test (non-parametric)
Comparing diagnostic accuracy	Sensitivity, specificity, predictive values AUROC
Comparing net benefit	Decision curve analysis
Estimating associations between exposure and binary outcomes	
Univariate analysis	Univariate logistic regression
Multivariable analysis	Multivariable logistic regression
Estimating differences in time-to-event outcomes	
Univariate analysis	Log rank test
Multivariable analysis	Cox regression

AUROC: Area under ROC curve.

Descriptive statistics

All studies include descriptive statistics. Whether data were normally distributed was investigated initially graphically by means of histograms. The central tendency and variation of normally distributed data are presented as means ± standard deviations (SD). Non-normally distributed data were presented as medians with min-max or inter quartile range (IQR). Binary and categorical data were presented as numbers and proportions.

Comparing groups

All studies also include group comparisons, either by exposure, for example allergy status and levels of HCC, or outcome, for example appendicitis/no appendicitis and uncomplicated/complicated appendicitis. Different statistical tests for comparing two

or more groups were used to evaluate the possible statistical significance of the differences between the means, medians, or frequencies of the groups.

Evaluating and comparing diagnostic accuracy

In Paper I the diagnostic performance was evaluated for four different clinical prediction scores. Sensitivity, specificity, positive and negative predictive values, rates of missed appendicitis (false negatives) and of negative appendectomies (false positives) were calculated according to each scoring systems cut-off levels for low, intermediate, and high risk of appendicitis. Sensitivity and specificity refer to the true positive and true negative rates, respectively. The positive and negative predictive values represent the probabilities that a subject with a positive or negative test truly has or has not got the disease, in this case appendicitis.

Table 6. A diagram showing how the diagnostic values used in the thesis were calculated, and how they are related

	Appendicitis	**No appendicitis**
Positive test	A	B
Negative test	C	D

Sensitivity = A / (A+C)
Specificity = D / (B+D)
PPV = A / (A+B)
NPV = D / (C+D)

A: True positive; B: False positive; C: False negative; D: True negative; PPV: Positive predictive value; NPV: Negative predictive value.

Another test that was used in Paper I was the measurement of the area under the receiver operating characteristics (ROC) curve (AUROC). The ROC curve is a plot of the true positive rates (sensitivity) and the false positive rates (1 - specificity) for all potential thresholds of a diagnostic test. It is commonly used to determine a diagnostic test's most valuable threshold, that is to find the threshold that provides the highest rate of trues within the accepted rate of false positives. AUROC is a summary of the overall diagnostic accuracy of a test and is often used for comparisons of ROCs for different diagnostic tests. The AUROC can range from 0–1, and a value of 0.7–0.8 is considered acceptable, 0.8–0.9 is excellent and >0.9 is considered outstanding[185].

Finally, a decision curve analysis was used to further compare the diagnostic properties of the four clinical prediction scores in Paper I. This was created by plotting the net benefits of the prediction scores for all possible threshold probabilities between 70 and 100%. Net benefit was calculated through the formula: *(true positives/n) - ((false*

positives/n)(threshold probability/1-threshold probability))*. Every patient's predicted probability of appendicitis for each scoring system was estimated through logistic regression. The net benefit integrates the benefits of a correct diagnosis and the harms of a wrongful one (sending a sick patient home or a healthy one to surgery) on the same scale[186].

Logistic regression analysis

Papers II-V included analyses of associations between a dependent binary variable and one or more independent variables. Associations were presented as ORs using logistic regression, due to the binary nature of the outcome variables. Variables that were significantly associated with the primary outcome in the univariate analyses were included in the multivariable analysis. In two of the papers, forward (Papers II and III) and backward (Paper II) selection was used.

In Papers II and III, interaction terms were included to assess effect modification over different values of an independent variable. Overall effect modification was assessed using the Wald test (Paper II) and the likelihood ratio test (Paper III).

In Papers II and III, the robustness of the association models to potential unmeasured confounders was quantified using E values. An E value represents the effect size that an unmeasured confounder would need to have to explain away the association between the primary exposure and the outcome variable.

Different kinds of sensitivity analyses were used to assess the consistency of the association models. For example, in Paper II, subgroup analyses were conducted in only the children with histopathologically confirmed diagnoses. In Paper II, a sensitivity analysis including the number of primary care visits 12 months before the appendicitis episode was also conducted.

Survival analysis

In survival analysis, a method used in Paper III, the expected time to outcome event (in this case uncomplicated or complicated appendicitis) was analyzed. Kaplan-Meyer plots were generated to visualize the exact time to event or censoring for children with and without allergies (primary exposure). Censoring is when the outcome of interest for different reasons cannot be observed for some individuals during the selected study period. In Paper III, censoring was performed due to loss to follow up (death, migration, or colectomy for other reasons than appendicitis) and at end of study (when the event, appendicitis, had not occurred before the end of the study). Log rank tests

were used in combination with the time to event estimates from the Kaplan-Meier plots to assess univariate differences between the groups. Cox proportional hazard regression was used to assess the multivariable association between the primary exposure and risk of outcome and reported as hazard ratio (HR). The HR compares the difference in the probability of the outcome occurring if the primary exposure is present or not.

Software

Primary handling of data was performed by means of Microsoft Excel for Mac (versions 11 and 16, Microsoft Corp, Redmond, WA, United States). Most statistical analyses in Paper I and all statistical analyses in Papers IV and V were carried out in IBM SPSS for Macintosh (versions 24 and 25, IBM Corp, Armonk, NY, United States). All statistical analyses in Paper II were performed in Stata/SE (version 14.1 for Windows, Stata Corp LP). All statistical analyses in Paper III were performed in R version 3.5.1 (R foundation for statistical computing, Vienna, Austria). R was also used to generate the decision curves and ROC plots in Paper I.

The decision curve-analyses in Paper I were performed by an external statistician. The same statistician was also consulted regarding the statistical methods in Papers IV and V.

Ethical considerations

All studies were approved by the regional ethics board, Etikprövningsmyndigheten, Lund, Sweden; DNR 2010/49 and 2013/614 for Paper I, 2010/49 for Paper II, 2014/856 for Paper III, 2017/242 for Paper IV, and 2013/614 for Paper V. Before inclusion in the prospective clinical studies (Papers I, IV and V) both (if two) parents or guardians received written and oral information before leaving their written consent to participation. Children were informed primarily orally, but written information could be given to older children. Children who verbally or physically (in the case of hair sampling in Paper IV) opposed study participation were excluded. All data were anonymized before statistical analyses, and the results were presented in such ways that make it impossible to identify single patients.

Main findings

Paper I

In the high-risk group, AIR score and pARC had substantially higher specificity and positive predictive value compared to the PAS and Alvarado score. Basing clinical decisions based solely on the AIR score and pARC would also result in fewer cases of negative appendectomies compared to the other two scoring systems (7% and 2% compared to 36% and 28%, p<0.001). In the low-risk group, the sensitivity, negative predictive value as well as rates of missed appendicitis were similar for all four scoring systems (Table 7).

Table 7. Diagnostic values and clinical outcome of prediction scores for pediatric appendicitis according to the published cut-off points for all cases of appendicitis

	PAS	AIR		Alvarado		pARC	
		Low	High	Low	High	Low	High
Sensitivity	95.3 (90.3-98.0)	88.1 (81.6-92.6)	27.8 (21.0-35.8)	96.7 (92.0-98.8)	84.1 (77.1-89.4)	97.2 (91.5-99.3)	39.8 (30.7-49.7)
Specificity	51.5 (43.7-59.2)	77.8 (70.6-83.7)	98.2 (94.4-99.5)	33.5 (26.5-41.3)	70.1 (62.4-76.8)	41.3 (31.3-52.1)	98.9 (93.2-99.9)
PPV	64.0 (57.3-70.2)	78.2 (71.1-84.0)	93.3 (80.7-98.3)	56.8 (50.5-62.9)	71.8 (64.4-78.1)	66.0 (58.1-73.2)	97.7 (86.5-99.9)
NPV	92.5 (84.6-96.7)	87.8 (81.2-92.4)	60.0 (54.0-65.9)	91.8 (81.2-96.9)	83.0 (75.5-88.6)	92.7 (79.0-98.1)	58.3 (50.2-66.1)
Missed appendicitis	7 (8)	18 (12)		5 (8)		3 (7)	
No appendicitis	81 (36)		3 (7)[a]		50 (28)		1 (2)[b]
Negative appendectomy	8 (5)		0 (0)		3 (2)		1 (2)

Sensitivity, specificity, PPV and NPV presented as % (95% confidence interval), missed appendicitis and no appendicitis presented as n (%). [a]p<0.05 when comparing the AIR score to the PAS and Alvarado score through Chi2 test.[b]p<0.05 when comparing the pARC to the PAS and Alvarado score. PPV: positive predictive value, NPV: negative predictive value; PAS: Pediatric Appendicitis Score, AIR: Appendicitis Inflammatory Response, pARC: pediatric Appendicitis Risk Calculator. PAS: low=0-5 and high 6-10; AIR score: low=0-4 and high=9-12; Alvarado score low=0-4 and high=7-10; pARC: low=0-14% and high 85-100%.

AUC values from the ROC curves were high for all scoring systems, both in the total cohort and the cohort with complicated appendicitis only. In the total cohort, values ranged from 0.90 for pARC and 0.86 for Alvarado score. In the complicated

appendicitis group, values ranged from 0.94 for AIR score and 0.91 for PAS and Alvarado score (Figure 8).

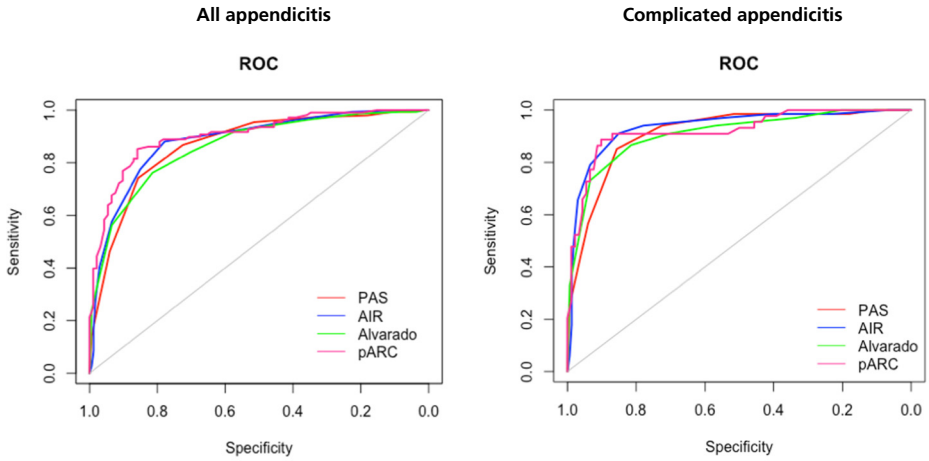


Figure 8. Receiver operating characteristics for appendicitis and complicated appendicitis for different clinical prediction scores in 318 children with suspected appendicitis. PAS: Pediatric Appendicitis Score, AIR: Appendicitis Inflammatory Response, pARC: pediatric Appendicitis Risk Calculator.

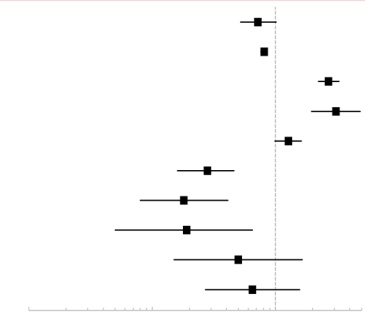
Decision curve analysis showed that in patients aged 0-15 years the net benefit was better for AIR score compared to PAS and Alvarado at most threshold probabilities. When including only children aged 4–15 years, and thus also pARC, pARC displayed the highest net benefit at almost all threshold probabilities.

Paper II

Complicated appendicitis occurred in 19.6% of the children with IgE-mediated allergy, compared to 46.9% of the non-allergic children ($p<0.001$). In the univariate logistic regression analysis, allergy was significantly associated with a lower risk of complicated appendicitis (OR 0.28 [95% CI 0.16-0.46], $p<0.001$). Younger age, longer symptom duration and presence of an appendicolith were associated with an increased unadjusted risk of complicated appendicitis (Table 8). In the multivariate logistic regression, allergic children had a three times lower risk of complicated appendicitis compared to children without allergy (aOR 0.33 (95% CI 0.18-0.59), $p<0.001$). In the subgroup analysis according to allergens, the lower adjusted risk remained for pollen allergy (aOR 0.29 (95% CI 0.12-0.69), $p=0.006$) and for fur or mite allergy (aOR 0.25 (95% CI 0.07-0.94), $p=0.04$), but not for egg or milk protein allergy and antibiotic allergy (Table 9).

Table 8. Unadjusted independent variables of complicated disease among 605 children undergoing surgery for acute appendicitis

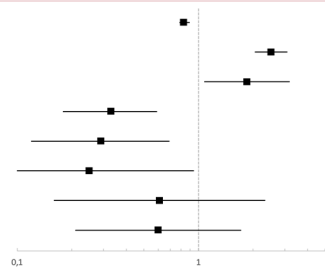
Variable	Uncomplicated appendicitis	Complicated appendicitis	OR (95% CI)	Uncomplicated appendicitis	Complicated appendicitis	p-value
Sex (male)	231 (66.2)	150 (58.6)	0.72 (0.52-1.01)			0.06
Age (years)	11 (9-13)	8 (4-11)	0.81 (0.76-0.85)			<0.001
Symptom duration (days)	1 (<1-1)	2 (1-3)	2.70 (2.23-3.26)			<0.001
Appendicolith	34 (9.7)	64 (25.0)	3.09 (1.96-4.86)			<0.001
No. of primary care visits last 12 mo[a]	0 (0-2)	1 (1-3)	1.27 (<1.00-1.62)			0.05
Allergy	83 (23.5)	20 (7.8)	0.28 (0.16-0.46)			<0.001
Pollen	44 (12.6)	7 (2.7)	0.18 (0.08-0.41)			<0.001
Fur/mite	18 (5.2)	3 (1.2)	0.19 (0.05-0.65)			0.008
Egg/milk protein	9 (2.6)	4 (1.6)	0.50 (0.15-1.65)			0.26
Antibiotic	14 (4.0)	8 (3.1)	0.65 (0.27-1.57)			0.34



Values presented as median (IQR) and as absolute number and percentage of patients; n(%). Univariate logistic regression was used with odds ratios (ORs) and 95% CIs. IQR: interquartile range. [a]Data were available for 95 patients (2015-2016).

Table 9. Adjusted variables for complicated disease among 605 children undergoing surgery for acute appendicitis

Variable	Adjusted OR (95% CI)	Uncomplicated appendicitis	Complicated appendicitis	p-value
Age (years)	0.83 (0.79-0.89)			<0.001
Sympton duration (days)	2.52 (2.06-3.08)			<0.001
Appendicolith	1.85 (1.08-3.17)			0.03
Allergy	0.33 (0.18-0.59)			<0.001
Pollen allergy[a,b]	0.29 (0.12-0.69)			0.006
Fur or mite allergy[a]	0.25 (0.07-0.94)			0.4
Egg or milk protein allergy[a]	0.61 (0.16-2.33)			0.47
Antibiotic allergy[a]	0.60 (0.21-1.71)			0.34



Values presented as median (IQR) and as absolute number and percentage of patients; n(%). Multivariable logistic regression was used with adjusted odds ratios (aORs) and 95% CIs. [a]Indicates compared with no allergy. [b]Includes allergies to allergens with cross-reactivity to pollen. The estimated E value for unmeasured confounding was 2.9 (95% CI 1.9-4.1).

Paper III

In the cross-sectional analysis, complicated appendicitis occurred in 12.8% of the allergic children, compared with 18.9% of the children without allergy (p<0.001). IgE-mediated allergy at the time of the appendicitis episode was associated with a lower absolute risk (13% [95% CI 11-14] versus 19% [18-20]), and lower unadjusted odds (OR 0.63 [95% CI 0.53-0.75]) for complicated appendicitis. The protective association remained after adjustment for age, sex, and parental education (aOR 0.80 [95% CI 0.67-0.96], p=0.021).

Seasonal antigen exposure was associated with a lower adjusted odds for complicated appendicitis (aOR 0.78 [95% CI 0.66-0.92], p=0.004), and ongoing antihistamine medication with a higher adjusted odds for complicated appendicitis (aOR 2.28 [95% CI 1.21-4.28], p=0.012).

Children diagnosed with IgE-mediated after their appendicitis episode did not have lower odds of complicated appendicitis compared to never allergic children (aOR 0.94 [95% CI 0.08-1.10], p=0.414).

In the longitudinal analysis, the risk of complicated appendicitis among the allergic children was reduced by one-third (incidence rate [IR] 0.13 vs 0.20 per 1000 person-years, HR 0.68 [95% CI 0.58-0.81], p<0.001). The risk for uncomplicated appendicitis did not vary with allergy status (IR 0.91 vs 0.91; HR 1.00 [95% CI 0.94-1.07], p=0.932) (Figure 9).

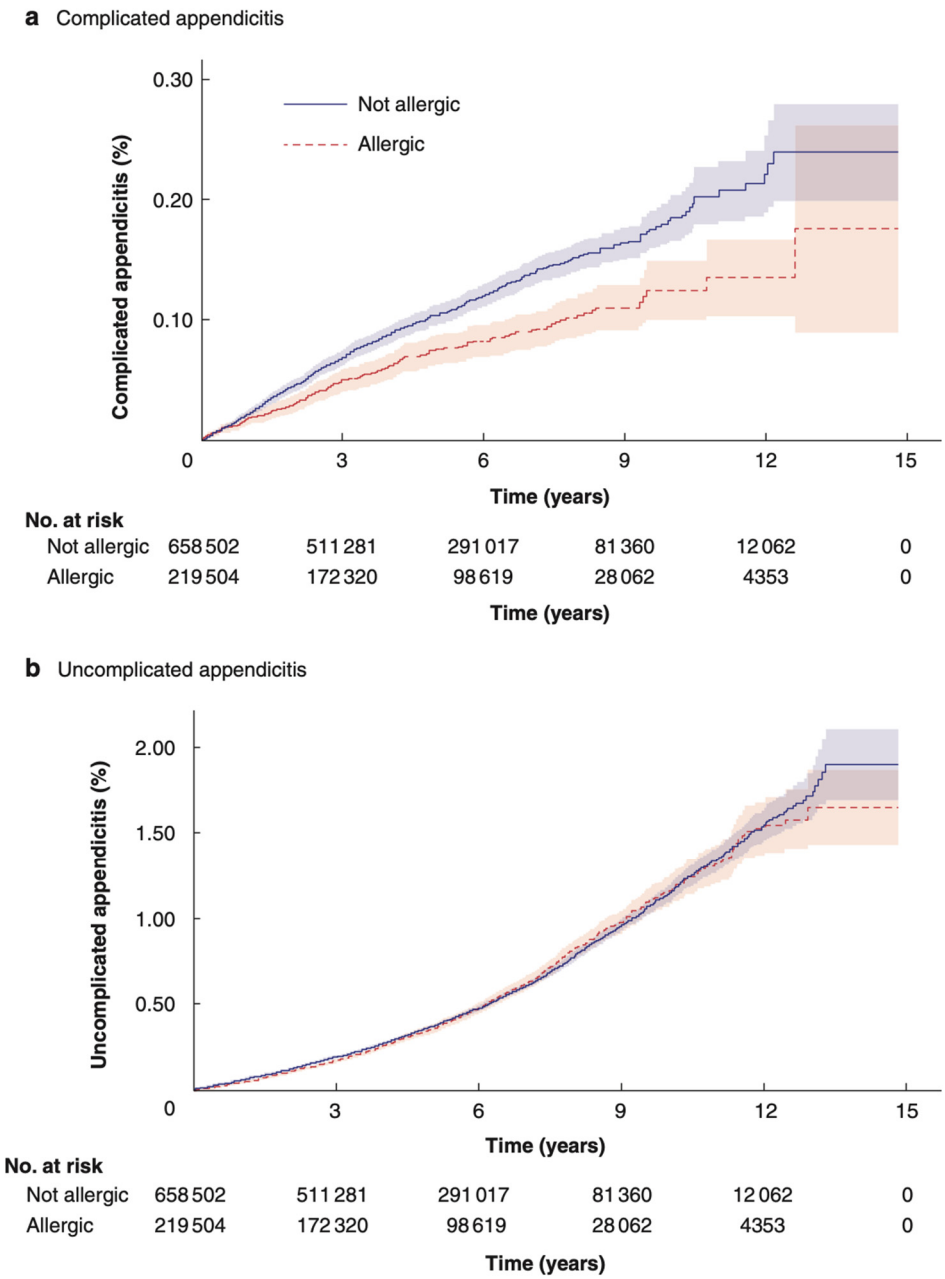


Figure 9. Complicated and complicated appendicitis during follow-up of 878,006 children with and without IgE-mediated allergy, 2000-2014. Incidence of a) complicated and b) uncomplicated appendicitis. Inclusion and matching occurred at the date of allergy diagnosis, and the date of exposure was therefore the starting point for time at risk rather than date of birth. Shaded areas represent 95% CIs.

Paper IV

In the univariate logistic regression analysis including both cases and controls, an increase in HCC between the measuring time points (4-6 and 0-3 months prior to inclusion) was associated with an increased risk of appendicitis (OR 7.57 [95% CI 2.49-22.67] p=0.001). This significant association remained in the multivariate analysis after adjustments for age, sex, and season (aOR 10.76 [95% CI 2.50-46.28], p=0.001) (Figure 10).

When only including children with appendicitis, high HCC 0-3 months prior to inclusion was associated with an increased risk of complicated appendicitis in the univariate analysis. This association was no longer significant in the multivariate analysis. An increase in HCC was not significantly associated with an increased risk of complicated appendicitis in the univariate analysis; however, it was significant in the multivariate analysis (aOR 7.86 (95% CI 1.20-51.63), p=0.03) (Figure 11).

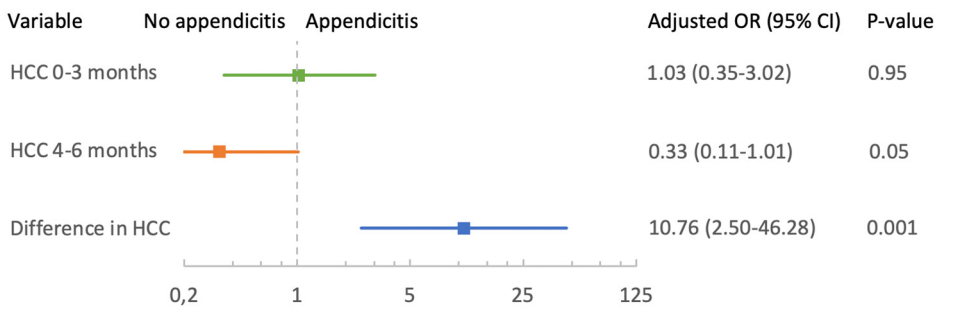


Figure 10. Adjusted odds ratios (ORs) for appendicitis for different timepoints of HCC in 51 children with appendicitis and 82 controls. n = 106 (66 controls and 40 cases) for HCC 4-6 months and difference in HCC. Adjusted for age, sex, and season.

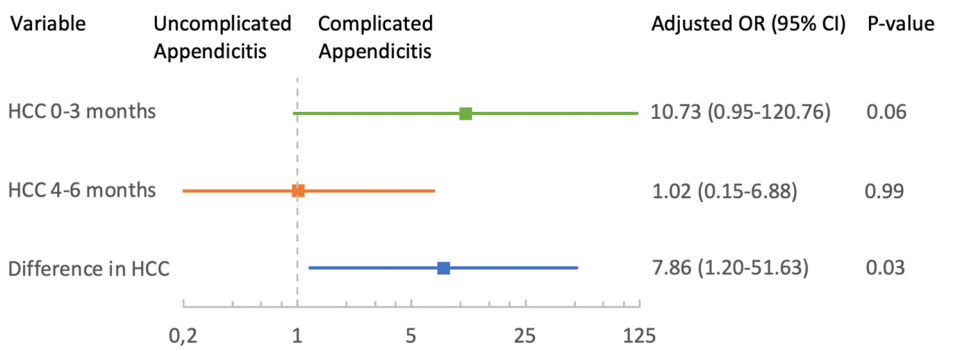


Figure 11. Adjusted odds ratios (ORs) for complicated appendicitis for different timepoints of HCC in 14 children with uncomplicated appendicitis and 27 children with complicated appendicitis. n = 40 for HCC 4-6 months and difference in HCC. Adjusted for age and sex.

Paper V

Concentrations of IL-9 and IL-13 were significantly higher in children with complicated appendicitis (1.8 [IQR 1.1-3.2] pg/ml and 24.6 [IQR 12.9-58.5] pg/ml) compared to the children with uncomplicated appendicitis (1.4 [IQR 0.8-2.1] pg/ml and 14.6 [IQR 10.2-24.3], p=0.047 and 0.002, respectively).

High concentrations of IL-13 were associated with an increased risk of complicated appendicitis, both in the univariate (Table 10) and multivariate (Table 11) analyses, after adjustment for age, symptom duration and presence of an appendicolith (aOR 1.02 [95% CI 1.01-1.04], p=0.011). Serum concentrations of total IgE, IL-4 and IL-19 did not significantly affect the risk of complicated appendicitis.

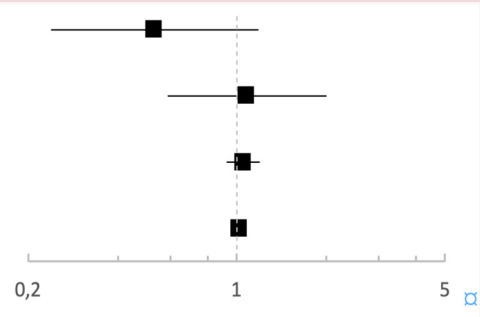
Table 10. Unadjusted independent variables of complicated appendicitis in 138 children with appendicitis

	Uncomplicated appendicitis (n=80)	Complicated appendicitis (n=58)	OR (95% CI)	p-value
Age (years)	11 (9-13)	9 (7-12)	0.84 (0.75-0.94)	**0.003**
Sex (male)	49 (61%)	38 (66%)	1.20 (0.60-2.43)	0.608
Allergy	15 (19%)	5 (9%)	0.41 (0.14-1.20)	0.103
Symptom duration				
0-24 h	42 (53%)	18 (31%)	Ref	Ref
24-48 h	27 (34%)	23 (40%)	1.99 (0.91-4.35)	0.086
48-96 h	10 (13%)	14 (24%)	3.27 (1.22-8.71)	**0.018**
>96 h	0 (0%)	2 (3%)	N/A	N/A
Season				
Spring	16 (20%)	17 (29%)	Ref	
Summer	14 (18%)	15 (26%)	(0.37-2.74)	0.987
Autumn	20 (25%)	12 (21%)	0.57 (0.21-1.52)	0.257
Winter	30 (38%)	14 (24%)	0.44 (0.17-1.12)	0.084
Appendicolith	13 (16%)	21 (36%)	2.86 (1.28-6.41)	**0.011**
Serum IgE elevated*	39 (49%)	21 (34%)	0.60 (0.30-1.19)	0.144
Serum IL-4 (pg/mL)	0.33 (0.21-0.62)	0.36 (0.20-0.70)	1.23 (0.69-2.19)	0.476
Serum IL-9 (pg/mL)	1.35 (0.83-2.10)	1.78 (1.10-3.17)	1.08 (0.96-1.22)	0.192
Serum IL-13 (pg/mL)	14.60 (10.15-24.34)	24.60 (12.91-58.53)	1.02 (1.01-1.04)	**0.005**

Values presented as median (IQR) and as absolute number and percentage of patients; n(%). Univariate logistic regression was used with odds ratios (ORs) and 95 % confidence intervals (95%CI). *Above reference intervals according to age. Symptom duration n=79 and 57; S-IL-4 n = 72 and 50, since some values were unmeasurable; S-IL-9 n = 78 and 55; S-IL-13 n = 78 and 57.

Table 11. Adjusted variables for complicated appendicitis in 138 children with appendicitis

	aOR (95% CI)	Uncomplicated appendicitis	Complicated appendicitis	p-value
Serum IgE elevated*	0.53 (0.24-1.18)			0.121
Serum IL-4 (pg/mL)	1.08 (0.59-1.99)			0.810
Serum IL-9 (pg/mL)	1.05 (0.93-1.19)			0.455
Serum IL-13 (pg/mL)	1.02 (1.01-1.04)			**0.011**



Multivariable logistic regression was used with adjusted odds ratios (aORs) and 95% confidence intervals (95% CI). *Above reference intervals according to age. Adjusted for age, symptom duration and presence of appendicolith.

Discussion

Diagnosis based on more than gut feeling

Even though appendicitis is the most common condition requiring acute abdominal surgery in children, only 1-9 % of children presenting to the pediatric ED with abdominal pain have appendicitis[94,187,188]. Confirming or dismissing the diagnosis of appendicitis in a child with abdominal pain can be difficult. Standard laboratory tests available to date have shown unsatisfactory diagnostic performances when used alone, but when incorporated in clinical prediction scores along with other anamnestic and clinical parameters the risk of appendicitis can be better assessed. The use of radiology has become increasingly popular, and it has been shown that imaging enhances the performance of clinical prediction scores[189,190]. However, which patients should undergo diagnostic imaging should be chosen with great care. Being too generous with imaging studies could result in diagnosing some children with mild symptoms and possibly self-limiting uncomplicated appendicitis.

Since appendicitis has long been considered a progressive disease, always resulting in perforation if left untreated, the clinical management has focused on prompt surgery to prevent perforation[73]. This, in turn, has led to an acceptance of high rates of negative appendectomies. More recent studies have indicated that perforation often occurs prehospitally, and that it can be hard to prevent[73]. At the same time, an in-hospital delay of up to 24-36 hours does not seem to increase the risk of perforation[147]. It has therefore been advocated that the focus should be on identifying children with a high risk of perforation and to diagnose and treat these patients as a matter of urgency. Children in whom there is a high suspicion of uncomplicated appendicitis (or low suspicion of complicated appendicitis), however, do not have to be rushed to surgery. In the future, they might not have to undergo surgery at all[149].

Diagnostic aids that can distinguish both appendicitis from other causes of abdominal pain, as well as complicated from uncomplicated appendicitis, would be much welcome additions to the diagnostic toolbox – both in future research on, for example, non-operative management of uncomplicated pediatric appendicitis and in clinical practice.

In Paper I, the diagnostic performances of four different clinical prediction scores for pediatric appendicitis were evaluated. This was the first prospective comparison of the PAS, AIR score, Alvarado score and the pARC in a pediatric population. The AIR score and the pARC had an overall higher diagnostic accuracy compared with the PAS and Alvarado score. Based on the results from this paper, we recommend the use of the AIR score and pARC over the PAS and Alvarado score when evaluating a child with suspected appendicitis. Diagnostic imaging should be limited to the children stratified to the intermediate risk-group according to the AIR score or pARC. An alternative or complement to imaging is active observation and repeated scoring.

The perfect clinical predictor is easy to apply in the clinical setting and can predict the outcome of interest accurately[191]. According to the results from Paper I, both the AIR score and the pARC accurately predicted the outcome of interest – appendicitis. However, the pARC was developed more recently than the AIR score, and has consequently been validated fewer times than it. Both these clinical prediction scores include variables of history, clinical findings, and results of standard laboratory tests (CRP, WBC, and ANC). At least at the pediatric ED at Skåne University Hospital, all these laboratory tests are analyzed routinely in pediatric patients with suspected appendicitis, readily available for incorporation in the prediction score calculations. A strength of the AIR score over pARC is that it can also be used on adult patients. However, at the ED at Skåne University hospital, ANC is not measured routinely in adult patients with abdominal pain, slightly complicating the implementation of the AIR score. Another advantage of the AIR score over the pARC, is that the pARC requires the use of an online-based or downloaded risk calculator[134], while the AIR score needs no such requirements – one just totals the scores for each included variable[131]. It has also been shown that the physician's clinical experience does not affect the diagnostic accuracy of the AIR score[133].

In Papers II and V, we found significant associations between longer symptom duration and an increased risk of complicated appendicitis. This is a previously known association[192], and symptom duration is included as a variable in the pARC. Since the AIR score aims primarily to identify patients with a high risk of complicated appendicitis, one could argue that this variable should be added to the prediction score. Furthermore, this thesis provides novel findings of a protective association between IgE-mediated allergy and complicated appendicitis (Papers II and III) and an association between increased concentrations of IL-13 and complicated appendicitis (Paper V). Perhaps inclusion of allergy status and novel biomarkers, such as IL-13, could increase the diagnostic properties of the AIR score further.

In Papers II and V, we also found significant associations between the presence of an appendicolith and an increased risk of complicated appendicitis. This has also been

found in other studies, and an appendicolith is considered to be a well-known risk factor for complicated appendicitis. Appendicoliths are diagnosed through imaging studies or perioperatively and could possibly be added as a variable in a future prediction score including US studies.

When appropriately constructed and validated, clinical prediction scores have several theoretical advantages over human decision making. They can incorporate more predictors into the combined risk estimates and generate reproducible risk estimates that are not defiled with feelings and subjectivity[125]. But what is most important is that clinical prediction scores can be more accurate than clinical assessment alone. A Swedish multi-center study has shown that implementation of the AIR score seems to reduce hospital admissions, use of diagnostic imaging, unnecessary surgeries, and costs without an increase in "relapse appendicitis and catch-up appendectomies"[193]. Even so, it seems that clinical prediction scores for appendicitis have not gained ground in clinical practice to the same extent as other clinical prediction scores, for example the CHA_2DS_2-VASc predicting the risk of stroke and thromboembolism in patients with atrial fibrillation[194] or the Wells score estimating the probability of deep-vein thrombosis[195]. It has been proposed that resistance to adopting new clinical prediction scores could be due to unawareness of their diagnostic value, that they are conceived as not user friendly, and that there might be too many available tools assessing the same thing, leaving the clinician confused regarding which one to use[125]. Considering the abundance of clinical prediction scores assessing the risk of appendicitis in children and/or adults, one could argue that clinicians would be less resistant to adopting a clinical prediction score suitable for use on both adult and pediatric patients.

Allergic to complicated appendicitis?

The pathological mechanisms behind appendicitis and its different disease severities are still largely an enigma. Learning more about the physiological processes involved in both the origin of the inflammation as well as the spontaneous resolution in contrast to the progression to perforation is fundamental for future development of diagnostic aids and new treatment strategies. Furthermore, to date there are no known means by which adverse outcomes from appendicitis can be prevented.

Previous epidemiological studies have implied that a person's immune response is involved in driving the inflammation towards an uncomplicated or a complicated disease course, where the uncomplicated course is associated with a dominance of a Th2-dependent immune response, while complicated appendicitis is associated with a

Th1/Th17-dependent immune response. Do the results from this thesis support this theory? Yes! And also: No?

In Paper II we evaluated the association of IgE-mediated allergy, a condition mediated through Th2-mediated immune responses, and the risk of complicated appendicitis. We found a strong and previously unknown association of IgE-mediated allergies and a lower adjusted risk of complicated appendicitis, where the allergic children had a three times lower adjusted risk of complicated appendicitis compared to the non-allergic children.

This finding was followed up and evaluated further in Paper III, where the protective association between IgE-mediated allergy and complicated appendicitis was confirmed. In this study, the risk of complicated appendicitis in allergic children was reduced by a third. This was attributable to a reduction in the incidence rate of complicated appendicitis and not to an increased incidence rate of uncomplicated appendicitis. Furthermore, this protective association was found only in children who had an onset of allergy symptoms before their appendicitis episode and not in children who were diagnosed with allergy afterwards. This further indicates that the mechanisms involved in the protective association of allergy on complicated appendicitis are in fact attributable to different kinds of immune responses, rather than to a genetic predisposition. Another interesting finding in Paper III was that seasonal antigen exposure during March to August was associated with a reduced adjusted risk of complicated appendicitis, while ongoing antihistamine treatment, defined as prescription of such drugs within 30 days before the appendicitis diagnosis, was in turn associated with an increased adjusted risk for complicated appendicitis. This indicates that the up- (antigen exposure) and down-regulation (antihistamine medication) of the allergy associated immune responses affects the protective association of allergy with complicated appendicitis.

In a retrospective study from 2021, 134 appendix specimens were analyzed for prevalence of IgE-positive cells. The authors found a significant increase of IgE-deposits in phlegmonous appendices compared to incidentally removed appendixes, but not compared to gangrenous appendicitis and negative appendectomies[196].

The results from these studies strongly support the hypothesis of an association between a Th2-mediated immune response. Since they are observational studies, no absolute conclusions regarding the causal mechanisms can be drawn. To further examine this newly found association, we investigated the association of serum concentrations of IgE and Th2-associated interleukins in children with complicated and complicated appendicitis (Paper V). We hypothesized that high concentrations of these biomarkers would be associated with a decreased risk of complicated appendicitis. Instead, we

found a significant association between increased concentrations of IL-13 and an increased risk of complicated appendicitis. Serum concentrations of IgE, IL-4 and IL-9 were not significantly associated with the risk of complicated appendicitis.

A previous study investigating levels of the Th2-associated interleukins IL-4, IL-5, and IL-9 in appendicular lavage of 33 patients with appendicitis and eight with negative appendectomies, showed that concentrations of IL-4 were significantly higher in the phlegmonous appendixes compared to the normal appendixes, but not compared to the gangrenous ones. No significant differences were found regarding the other interleukins[197].

Since blood samples were collected at the time of evaluation at the pediatric ED, and the concentrations of IgE and interleukins are affected by acute inflammation, one hypothesis is that the high concentrations of IL-13 reflect an attempt by the body to slow down a major inflammatory response in complicated appendicitis.

The adjusted odds of complicated appendicitis with IgE concentrations above the reference intervals (aOR 0.53 [95% CI 0.24-1.18]) indicated that high levels of IgE might be a predictive factor of uncomplicated appendicitis. Since Paper V included substantially fewer patients compared to Papers II and III, it is possible that this non-significant association is the result of a power issue.

The hairy connection between appendicitis and stress

Stress has been associated with several diseases, primarily depression or anxiety[198], and cardiovascular diseases[199,200]. It has also been associated with intestinal diseases, such as inflammatory bowel disease and irritable bowel syndrome[201].

Stress increases the activity of the HPA axis, which, in turn, increases the secretion of cortisol. Cortisol suppresses the Th1-mediated immune responses primarily, creating a shift towards the Th2-mediated immune responses[180]. We therefore hypothesized that high levels of hair cortisol would be associated with a decreased risk of complicated appendicitis.

Increased stress was associated with an increased risk of appendicitis and complicated appendicitis. High levels of HCC months 0-3 before inclusion were associated with higher odds for complicated appendicitis; however, this finding was not significant. The finding of an association between stress and increased risk of complicated appendicitis was in contrast to our hypothesis. Since this was an observational study evaluating associations, we can only speculate on the causal mechanisms behind the

results. One possible explanation is that stress increases the intestinal permeability, increasing the risk of bacterial translocation into the intestinal wall[201]. It has also been suggested that long-term stress and a subsequent prolonged elevation of cortisol secretion might lead to a downregulation of the cortisol receptors on leukocytes, making them less susceptible to anti-inflammatory signals. This, in turn, would allow the inflammatory process to flourish[178]. One could also speculate that high HCC in this study might be reflective of a low socioeconomic status[202,203] – a factor that was not controlled for. In Sweden, parental unemployment was associated with an increased risk of complicated appendicitis[38].

To the best of our knowledge, this is the first time the association between stress and appendicitis has been studied, and these results must be validated before any firm conclusions can be drawn. In our study we focused on measuring HCC reflecting the activity of the HPA axis during the last 6 months before inclusion, in order not to exclude too many children due to them having hair shorter than 6 cm. To evaluate the effects of increased or decreased stress on the risk of appendicitis and complicated appendicitis, we segmented the hair samples into two sections measuring 3 cm each, reflecting the activity of the HPA axis during months 0-3 and 4-6 before inclusion. The stress load is still measured at a rather blunt timeline, and we cannot know if when during months 0-3 the stress was increased. Since this was a rather exploratory study, one could argue that the hairs should have been segmented into shorter sections, for example six segments of 1 cm each. Unfortunately, such small hair segments would be of insufficient weight for the analyses to be reliable.

Methodological considerations

This thesis includes different methods of evaluating the inflammatory responses in pediatric appendicitis, from translational and clinical studies to national registry-based epidemiological studies. However, the results of all studies must be interpreted in the light of their limitations.

All studies are observational – a method considered inferior to the randomized study design. In these cases, however, the predictors of interest (allergy, stress, and serum biomarkers) could not be randomized. Theoretically one could subject children to long-term stress, but this would be highly unethical. In the case of Paper I, we could have considered a randomized study design, where the clinical management would be based solely on the results from one of the different scoring systems and possibly the clinical assessment of a senior pediatric surgeon in a fifth arm.

Observational studies cannot be compared to randomized studies when it comes to drawing conclusions regarding causality. And in much research, including my own, the questions often evolve around causality, for example: *"Does allergy cause a lower risk of complicated appendicitis?"*. When conducting observational research, it is therefore important to acknowledge and handle as much of the potential biases and confounders as possible, to produce estimates of the associations between exposures and outcomes that are as representative of the true effect as possible. One advantage of observational studies, especially register-based studies, is that they assess the reality as it is, without the influence of an experimental study design.

Hypothesis testing

In statistical hypothesis testing, different statistical methods are used to either reject or accept a null hypothesis (usually stating that there is no difference between the studied groups) based on whether the probability that the observed results would occur if the null hypothesis were true is over or below a pre-specified threshold (the significance level). In all statistical hypothesis testing, there is a risk of type I errors – the mistake of rejecting a null hypothesis that is actually true - and type II errors – the mistake of accepting a null hypothesis that is actually false[204]. While type I errors are associated with the significance level, type II errors are associated with the analyses' power. Statistical power is related directly to statistical significance level, effect size and sample size, but can be influenced by other factors, such as variability, measurement errors and confounding factors. The risk of type II errors can be minimized by performing *a priori* power analyses, to determine the required sample size. Power analyses were not performed in studies IV and V since we, due to the exploratory study design, had no prior estimates of expected changes in HCC and the interleukins.

Type II errors possibly occurred in studies IV and V. In study IV, for example, high levels of HCC in months 0-3 before appendicitis were not significantly associated with an increased risk of complicated appendicitis, even though the 95% CI of the OR almost did not span over 1, and the p-value was close to our pre-determined significance level. In Paper V, known risk factors of complicated appendicitis, such as longer symptom duration and the presence of an appendicolith, were not significantly associated with an increased risk of complicated appendicitis in the multivariable analysis. These kinds of results do not occur seldomly and are approached in different ways. In these circumstances. it is important to acknowledge that the results might be hampered by a power issue, and may not be too aggressive in confirming potentially false null hypotheses.

Validity, bias, and confounding

The concept of validity applies to all clinical studies and includes the two domains internal and external validity. Internal validity corresponds to the extent to which an observed effect corresponds to the true effect in the studies population, and not methodological mishaps. External validity, on the other hand, refers to the generalizability of the observed effects – are they true in real life, or at least in the sample the study population is thought to represent? The results from clinical studies can be affected by the effects of confounders and different types of biases.

Bias

Studies I, IV and V were prospective clinical studies, in which the study population was well defined, and data were registered in a study matrix, reducing the risk of selection and reporting bias, respectively. Furthermore, in the prospective studies, the appendicitis diagnosis and disease severity were confirmed through histopathological examination, decreasing the risk of bias due to misclassification in the outcome variable – a strength in terms of internal validity. Due to the retrospective design of study II, histopathology reports were not available for all patients, indicating a potential misclassification bias. This was investigated through a sensitivity analysis of only the patients with histopathologically confirmed diagnoses, where the investigated association was even stronger than in the total cohort.

In Paper IV we had a high proportion of complicated appendicitis, probably due to a selection bias of the most ill patients, since we did not manage to include some of the patients who were discharged rapidly after an uncomplicated appendectomy. This surely affects the external validity of the study.

Another potential threat to the external validity is related to the study settings of Papers I and IV, where we only included children who had been referred to the on-call pediatric surgeon for clinical evaluation at the pediatric ED. This means that the results might not be applicable in other settings, such as in primary care. This is especially important to note for Paper I, since the prevalence of a disease greatly affects the predictive values of a test: with decreased prevalence the PPV decreases while the NPV increases.

In Paper III, however, the risk of selection bias is considerably low, since it included a total population of children with free access to healthcare meticulously monitored in national registers. However, being a register-based study, it entails a risk of reporting bias of, for example, allergy status, where children with mild symptoms might not seek medical care to obtain a diagnosis or prescribed allergy medication. Furthermore, there is a high risk of misclassification bias of the appendicitis severity, especially in the

longitudinal analysis since the ICD-10 codes for appendicitis were revised in 2010, now focusing on clinical presentation (no, local, or general peritonitis) instead of the presence of perforation or an abscess. If this would cause a decrease in the proportion of reported rated of complicated appendicitis, we do not believe that this would have a significant effect on the main outcome.

Confounding

Confounding means that the effect of the exposure of interest is mixed with the effect of another variables, a confounder[205]. Confounders must be associated with the exposure as well as the outcome, but not in the causal pathway between the two[205].

To minimize the influence of potential confounders in our results, multivariable logistic regression, including many known risk factors of appendicitis and complicated appendicitis, was used in Papers II-V. These variables were chosen through univariate logistic regression, where the variables significantly associated with the primary outcome were included in the multivariable analysis. This is a common way of deciding which variables (possible confounders) to adjust for, but it might be hampered with problems when interpreting the results. One must always consider how the potential confounders interact with the effect of interest when choosing covariates, since the introduction of variables that do not causally act as confounders of the studied effect might introduce random effects, which can potentially influence the results.

Another potential source of error in observational studies is the effect of unmeasured confounding. In Papers II and III, the robustness of the association between IgE-mediated allergy and complicated appendicitis in relationship to potential uncontrolled confounders was evaluated through a measurement of the E-value. The E-value was introduced in 2017 and is defined as the minimum strength of the association between an uncontrolled confounder and both the exposure and outcome of interest necessary to trivialize the specific association. It is intended for use in observational studies evaluating causality[206]. The E-value should, however, not be seen as an alternative to a careful consideration of and adjustment for measurable confounders and other potential biases[207]. Furthermore, there does not seem to be a consensus regarding what constitutes a small or large enough E-value for the robustness of the studies association to be considered weak or strong, respectively, in relationship to uncontrolled confounding[207,208].

The importance of not comparing apples and oranges

A major issue within the research field of appendicitis is the vast heterogenicity in the classification of the disease and its different severities. In the clinical setting, the surgeon's perioperative macroscopic assessment of the degree of inflammation

determines the need for postoperative antibiotics and the length of hospital stay. In the research setting, misclassification makes it difficult to obtain reliable results and to compare research. Due to discordance between the perioperative and the histopathological assessment[68,69,209,210], histopathological examination of the appendixes could be considered to be the comparative standard in research. This was used in all clinical studies included in this thesis (Papers I, II, IV and V). Perforated appendicitis and appendicular abscess are considered clinical diagnoses, based on perioperative or radiological findings. However, opinions on what constitutes perforated appendicitis differ. Some studies include gangrene and purulent fluid in the abdomen in the definition[211], while others use an evidence-based definition of a visual hole in the appendix or the finding of a fecalith in the abdominal cavity[72]. The rates of postoperative abscesses vary more in studies that do not use an evidence-based definition of perforated appendicitis, compared to the ones that do[211] – a finding that might be attributed to misclassification of patients with milder forms of appendicitis.

Etiology and pathogenesis – a summary

Looking back at the research questions that constituted the foundation for this thesis, it can be concluded that they have only to some extent been answered. And perhaps some of the results from this thesis raise more questions than they answer. The results do, however, offer new insights to the inflammatory processes that precede clinically manifested appendicitis in its different forms. Hopefully these insights can be used as clinical predictors by adding a few pieces to the large puzzle that is the diagnosis of appendicitis in children.

Most likely, appendicitis is a multifactorial disease, both regarding the initial triggering event and the subsequent inflammatory processes (Figure 12). Obstruction of the appendix lumen, often by an appendicolith, is likely the triggering event in many – but far from all – cases, especially the ones with a complicated disease course[53]. The incidence of appendicitis varies with age, and it is more common in males than females[32], suggesting age- and gender-related associations. Time and space clusters are suggestive of other causes, such as infections, but no particular agents have been identified[55]. Genetic and environmental factors have also been proposed[60,61]. This thesis adds increased stress as a potential predictor of appendicitis.

The disease severity also seems to be associated with many factors, such as age, sex, symptom duration[192], presence of an appendicolith[53,151], genetic factors, and socioeconomic factors[38]. This thesis adds allergy status and stress to this potpourri of prognostic factors for complicated appendicitis.

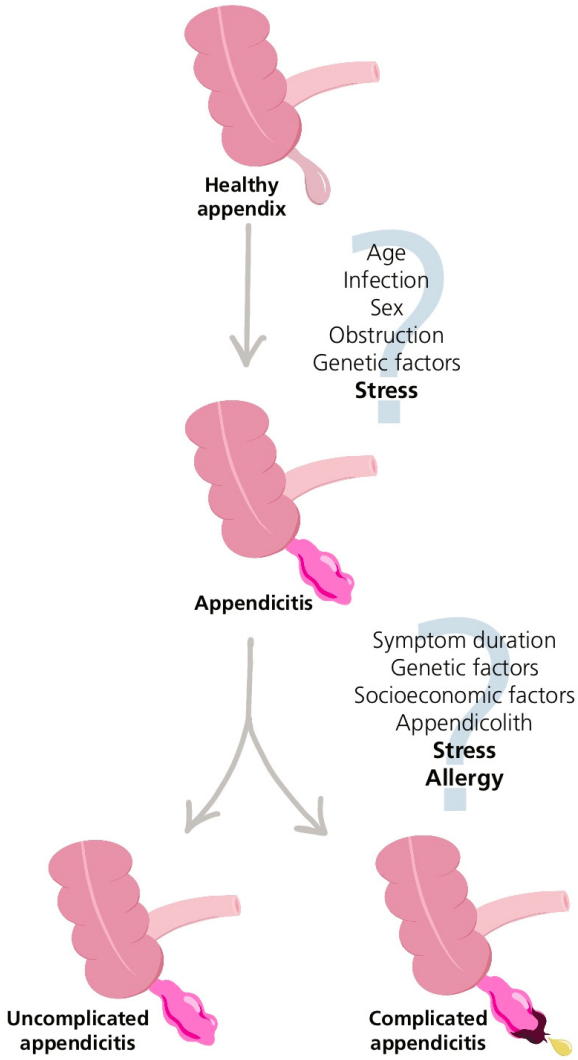


Figure 12. A summary of the prognostic and risk factors of appendicitis and complicated appendicitis, including the ones proposed by this thesis.

Conclusions

- The AIR score and pARC have a higher diagnostic accuracy of diagnosing appendicitis in children compared with the PAS and Alvarado score. To safely rule out the diagnosis of appendicitis based on the results from clinical prediction scores remains a challenge.
- Children with IgE-mediated allergy have a significantly reduced incidence of complicated appendicitis compared to non-allergic children. The incidence of uncomplicated appendicitis, however, is the same among allergic and non-allergic children. This supports the theory that uncomplicated and complicated appendicitis constitute two different disease entities, and that uncomplicated appendicitis is associated with a Th2-dominant immune response. Seasonal pollen antigen exposure was associated with a decreased risk of complicated appendicitis, whereas histamine medication was associated with an increased risk.
- An increased in HCC was associated with an increased risk of appendicitis and complicated appendicitis in children. This indicates that stress may modulate the inflammatory processes involved in the etiology and pathogenesis of pediatric appendicitis.
- Increased concentrations of serum IL-13 were associated with an increased risk of complicated appendicitis in children. Serum concentrations of total IgE, IL-4, IL-9 were not associated with an increased risk of complicated appendicitis.

Future aspects

Appendicitis is an enigmatic disease, and much regarding its etiology remains to be understood fully. The immunological processes involved in uncomplicated and complicated appendicitis should be evaluated further, for example by evaluating the inflammatory cells and mediators in the appendixes themselves. Furthermore, it would be interesting to further investigate the previously unknown association between stress and appendicitis – possibly by first validating the finding in other populations and throughout their life courses.

To provide the best possible care for children with appendicitis, much effort must be devoted to the ability to distinguish uncomplicated from complicated appendicitis – especially in an era when non-operative management for uncomplicated appendicitis is looking more and more appealing. Since allergy status was found to be such a strong prognostic factor for complicated appendicitis, its diagnostic properties should be evaluated, for example through incorporation in an existing prediction score. Additionally, new biomarkers for appendicitis and complicated appendicitis should be evaluated alone and in conjunction with clinical prediction scores. Well-validated clinical prediction scores for appendicitis should be used to a greater extent in the clinical practice.

An important keystone in all research, not least in the appendicitis field, is comparability. An international consensus regarding the classifications, both histopathological and clinical, of uncomplicated and complicated appendicitis is highly warranted.

Populärvetenskaplig sammanfattning

Bakgrund

Blindtarmsinflammation (appendicit) är ett mycket vanligt sjukdomstillstånd som drabbar ca 7–9 procent av befolkningen någon gång under deras livstid. Det kan uppstå när som helst under livet, men är som vanligast mellan 10 och 29 års ålder.

När man talar om sjukdomstillståndet på svenska är det lätt att man drabbas av begreppsrelaterad förvirring. Begreppet ”blindtarm” på svenska syftar egentligen till den första delen av tjocktarmen som börjar blint och sedan ansluter till slutet av tunntarmen. Denna del av tjocktarmen heter även cecum (vilket betyder ”blind” på latin), och det är här det maskformiga bihanget (appendix) sitter. Alltså skulle kanske ”blindtarmsbihangsinflammation” vara en mer korrekt svensk översättning av appendicit.

Trots att appendicit är ett väldigt vanligt tillstånd har man ännu inte helt kartlagt sjukdomsmekanismerna bakom. Länge har man trott att appendicit uppstår till följd av att någonting täpper till bihanget från insidan, vanligtvis en avföringssten eller svullen lymfvävnad, varefter bihanget svullnar och blir inflammerat. Någon tilltäppande process har dock bara kunnat påvisas i en del av, men långt ifrån alla, fallen.

Vidare har man länge betraktat appendicit som ett successivt progredierande tillstånd som oundvikligen leder till att bihanget brister (perforerar) om det lämnas utan behandling. Nu vet man att många fall av appendicit läker av sig själva, även utan behandling. Därför kategoriserar man ofta appendicit till antingen okomplicerad appendicit, som kan läka spontant utan behandling, och komplicerad appendicit, som riskerar att perforera om den lämnas obehandlad. Tidigare kliniska och epidemiologiska studier har indikerat att en individs eget immunförsvar kan vara involverat i att driva inflammationen mot ett okomplicerat respektive komplicerat sjukdomsförlopp. Dock kvarstår att kartlägga exakt vilka mekanismer som initialt triggar inflammationen och vad som driver den mot någon av de två typerna av appendicit.

Trots omfattande medicinska framsteg sedan den första blindtarmsoperationen (appendektomin) 1735, är det fortfarande ofta en klinisk utmaning att diagnostisera appendicit, i synnerhet hos barn. Buksmärta är en vanlig anledning till att barn söker

vård både på vårdcentraler och akutmottagningar. Bland dessa barn har de flesta ofarliga och självläkande tillstånd, men en betydande andel har sjukdomar som faktiskt kräver akut behandling, däribland appendicit. Jämfört med vuxna patienter är barn ofta svårare att bedöma eftersom både sjukdomshistorien och kroppsundersökningen kan vara mer svårvärderade. Detta resulterar i att en stor andel patienter får fel diagnos i den initiala handläggningen, vilket i fallet med appendicit kan leda till att man missar även en komplicerad appendicit, eller att man opererar barn och bihanget visar sig vara friskt. Båda dessa utfallen riskerar att orsaka onödig belastning för patienterna, i form av risk för onödig kirurgi samt för komplikationer till brusten appendicit, så som infektioner och varansamlingar i buken, såväl som för sjukvården. För att underlätta den kliniska handläggningen av barn med misstänkt appendicit har man utvecklad kliniska beslutsstöd i form av kliniska poängsystem som kombinerar parametrar från patientens sjukdomshistoria, den kroppsliga undersökningen och resultat från blodprover för att på ett mer objektivt sätt värdera risken för appendicit. Om risken värderas som låg eller medelhög kan man till exempel observera patienten, antingen i hemmet eller inneliggande på sjukhuset, eller utreda vidare med ultraljudsundersökning. Om risken värderas som hög, talar det för att man bör gå vidare med titthålskirurgi.

För närvarande finns inget blod- eller urinprov som med säkerhet kan skilja okomplicerad från komplicerad appendicit. Att vid den initiala bedömningen kunna skilja patienter med okomplicerad och komplicerad appendicit åt blir alltmer viktigt, sedan man börjat introducera icke-kirurgisk behandling (med antibiotika eller ingen behandling alls) som ett alternativ till appendektomi. Detta sker fortfarande inom ramen för kliniska studier, men kan komma att bli rutinbehandling för patienter med okomplicerad appendicit. Utmaningen blir att selektera fram just dessa patienter.

Syfte

Syftet med den här avhandlingen var att öka kunskapen kring de inflammatoriska mekanismerna kopplade till appendicit i dess olika former, samt att utvärdera befintliga och undersöka potentiella nya kliniska beslutsstöd (prediktorer) för okomplicerad och komplicerad appendicit.

Studie I-V

Studie I var en utvärdering och jämförelse av fyra olika kliniska poängsystem för appendicit hos barn. Vi fann att de två mest väletablerade poängsystemen för blindtarmsinflammation hos barn, Pediatric Appendicitis Score (PAS) och Alvarado score, hade lägre diagnostisk träffsäkerhet jämfört med de nyare och för barnpatienter mindre väletablerade Appendicitis Inflammatory Responce (AIR) score och pediatric Appendicitis Risk Calculator (pARC).

Studie II var en utvärdering av sambandet mellan allergi och komplicerad appendicit hos barn. Bakgrunden till denna studie var att tidigare studier funnit samband mellan okomplicerad appendicit och en viss typ av immunsvar, så kallat T-hjälparcell (Th) 2-medierat immunsvar. Baserat på att det Th2-medierade immunsvaret är ansvarigt för symtomen vid klassisk allergi, ville vi undersöka om barn med allergi löpte en lägre risk för komplicerad appendicit jämfört med icke allergiska barn. Vi fann att så var fallet; barn med allergi hade en tre gånger lägre risk för komplicerad appendicit jämfört med icke-allergiska barn.

Studie III var en nationell registerstudie som syftade till att verifiera och vidare utreda det skyddande sambandet mellan allergi och komplicerad appendicit. Vi fann att allergiska barn löpte en tredjedels lägre risk för komplicerad appendicit jämfört med icke-allergiska barn, medan risken för okomplicerad appendicit inte påverkades av allergi-status. Vi såg även att för att ha ett skydd mot komplicerad appendicit, måste allergin ha debuterat före appendiciten. Vidare fann vi att säsongsbunden exponering för allergiantigen (ämnen som utlöser allergiska reaktioner, till exempel pollen) också var en skyddande faktor, samt att pågående antihistaminbehandling (allergimedicinering) var en riskfaktor för komplicerad appendicit.

I studie IV undersökte vi om långvarig stress, vilket påverkar immunförsvaret, är kopplat till risken för appendicit och komplicerad appendicit hos barn. Vi undersökte stressnivåer hos barn med appendicit och friska kontroller genom att mäta koncentrationer av stresshormonet kortisol i hårprover. Detta är möjligt eftersom kortisolmolekyler lagras in i hårstråna när de växer från roten i koncentrationer som motsvarar de som cirkulerar i blodomloppet under tiden håret växer. Vi fann att en ökad biologisk stress, mätt som en ökning av hårkortisolkoncentrationerna de tre senaste månaderna före hårinsamlingen jämfört med de tre månaderna dessförinnan, var associerat med en ökad risk för appendicit och komplicerad appendicit.

I studie V ville vi vidare utreda sambandet mellan det Th2-medierade immunförsvaret och okomplicerad appendicit, och undersöka om någon biomarkör kopplad till den här typen av immunförsvar potentiellt skulle kunna användas som kliniskt beslutsstöd i framtiden. Vi fann att höga nivåer av ett av immunförsvarets signaleringsämnen, interleukin (IL)-13, var associerat med en ökad risk för komplicerad appendicit. Detta var oväntat, eftersom IL-13 är ett viktigt signaleringsämne för det Th2-medierade immunförsvaret.

Sammanfattning

Än återstår mycket att förstå om de många och förmodligen samverkande sjukdomsmekanismerna bakom appendicit, och hur man säkert och effektivt ska kunna

diagnosticera sjukdomstillståndet i dess båda former. Det den här avhandlingen bidrar med är ny och fördjupad kunskap kring de immunologiska mekanismerna kopplade till appendicit. Framför allt visar den på tidigare okända samband mellan allergi respektive stress och appendicit hos barn. Förhoppningsvis kommer kunskapen om dessa samband i framtiden kunna användas i kliniska sammanhang, för att kliniker på ett mer säkert och objektivt sätt ska kunna värdera risken för appendicit och komplicerad appendicit. Vidare bidrar avhandlingen med information om vilka av de befintliga kliniska poängsystemen som bör användas för att värdera risken för appendicit hos barn.

Acknowledgements

Jag vill börja med att rikta ett stort tack till de barn och föräldrar som deltagit i våra kliniska studier. Vidare vill jag tacka Lunds universitet, Södra Sjukvårdsregionen, Region Skåne och Region Östergötland för allt stöd.

Ensam är sällan stark, inte heller i arbetet med en avhandling. Jag är lyckligt lottad som fått hjälp och stöttning från fantastiska handledare, kollegor, familjemedlemmar och vänner. Ett särskilt tack till:

Lars Hagander, min handledare, för att du visat mig hur man är en bra handledare. Tack för att du är så klok och för att du outtröttligt ansträngt dig för att dela med dig av din kunskap och erfarenhet. Jag är tacksam för att du fått mig att se min egen progression under dessa år. Din förmåga att lyfta fram nya perspektiv och finurligheter har hjälp mig vidga min egen tankehorisont.

Martin Salö, min handledare, för att du med ett aldrig sinande engagemang guidat mig genom denna resa. Du är en sann naturkraft. Din förmåga att hålla många järn i elden är otroligt inspirerande, och jag hoppas kunna bli åtminstone hälften så effektiv som du. Även du är en mästare i handledning! Du får mig att känna ansvar och vara drivande, samtidigt som du alltid är tillgänglig för hjälp och stöttning.

Mina medförfattare, **Pernilla Stenström, Erik Omling, Niklas Rudolfson, Juan Merlo, Bodil Roth, Bodil Ohlsson, Elvar Theodorsson, Michaela Runnäs** och **Gustav Lovén**, för det fina samarbetet! Stort tack även till **Helena Quist** på Klinisk Kemi i Linköping för analys av hårkortisolkoncentrationerna, och till **Anna Åkesson** för hjälp med decision curve-analysen och för all övrig statistisk vägledning.

Alla fina **kollegor på barnkirurgen** och **barnakuten** i Lund för er fantastiska hjälp med rekrytering av patienter och insamling av blodprover. Tack för härligt sällskap på kongresser – i synnerhet på EUPSA i Paris 2018!

Mina fantastiska **kollegor på kirurgen i Malmö/Lund** för att ni sätter guldkant på varje arbetsdag och får mig att utvecklas som kirurg och kollega. Särskilt tack till min chef, **Tobias Axmarker**, för ditt viktiga engagemang i vår arbetsmiljö och för att du bidrar till att skapa en härlig sammanhållning bland oss ST-läkare. Tack även för att du sett till att jag kunnat fokusera helhjärtat på att ro denna avhandling i land.

Alla mina underbara **vänner**. Ni är allt!

Familjen Grünfeld, för att jag fått bli en del av er fina familj. För resorna och alla underbara middagar i Höllviken - välbehövda avbrott från arbetet med denna avhandling.

Mina syskon, **Kalle, Stein, Lena** och **Linda**. Jag är stolt över att få vara er stora- och lillasyster.

Min busiga, mysiga och på alla sätt underbara hund, **Olle**. Tack för ditt sällskap under alla timmar framför datorn. Nu kommer jag ha mycket tid över för bollkastning på Ribban!

Simon, min kärlek och bästa vän. Tack för att du är du, och för att du gör livet vi delar till en fröjd. Med dig har jag alltid roligt. Du är bäst!

Mina föräldrar, **Annette** och **Elvar**. Den här avhandlingen är tillägnad er, mina största förebilder. Tack för att ni alltid stöttar och uppmuntrar mig utan att någonsin ställa krav. Tack för ert outtröttliga lyssnande och för att ni är så kloka. Tack för att ni visar mig hur det känns att vara ovillkorligt älskad.

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Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

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Department of Clinical Sciences, Pediatrics

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:62
ISBN 978-91-8021-223-6
ISSN 1652-8220