Diploma Thesis

Exhaled Volatile Organic Compounds during Pediatric Open and Laparoscopic Appendectomy – A Prospective Randomized Pilot Study

Submitted by

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Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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List of Abbreviations

CRP:	C-Reactive Protein
KUS-Scale:	Kinder Unbehagen und Schmerzskala
NOTES:	Natural Orifice Transluminal Endoscopy Surgery
PAS:	Pediatric Appendicitis Score
ROS:	Reactive oxygen species
VOC:	Volatile organic compounds
WBC:	White Blood Cell Count

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Zusammenfassung

Einleitung: Die akute Appendizitis zählt zu den häufigsten Gründen eines akuten Abdomens im Kinder- und Jugendalter. Die Behandlung erfolgt durch eine offene oder laparoskopische Appendektomie, wobei derzeit noch unklar ist welche der zwei Methoden weniger oxidativen Stress auslöst. Die Analyse von flüchtigen organischen Verbindungen (volatile organic compounds, VOCs) aus der Ausatemluft bietet die Möglichkeit, den Verlauf des oxidativen Stresses intraoperativ nachzuverfolgen.

Methode: Im Rahmen dieser prospektiven randomisierten Pilotstudie wurden von 14 Kindern Proben der Ausatemluft während einer offenen (n=7) oder einer laparoskopischen (n=7) Appendektomie zu fünf verschiedenen Zeitpunkten (nach Narkoseeinleitung, 15 min, 30 min, 45 min nach Schnitt und nach Narkoseende) abgenommen. Als oxidative Stressparameter wurden die Substanzen Aceton, Isopren, Dimethylsulfid und Benzen gewählt und an der Technischen Universität Graz mittels Gaschromatographie und Massenspektrometrie analysiert.

Resultate: Es zeigte sich ein signifikanter Unterschied in der Acetonkonzentration zwischen den zwei Operationsmethoden beim Zeitpunkt der ersten (Narkoseinleitung) und zweiten (15 min nach Schnitt) Probenentnahme. Die Dimethylsulfidkonzentration war signifikant unterschiedlich zwischen den beiden Methoden während den ersten drei (nach Narkoseeinleitung, 15 min, 30 min nach Schnitt) und der letzten (nach Narkoseende) Probenentnahme. Isopren und Benzen zeigten keine signifikanten Unterschiede beim Vergleich der Abnahmezeitpunkte. Die Benzenkonzentration hatte jedoch einen signifikanten Verlaufsunterschied in der laparoskopischen Appendektomie, aber veränderte sich bei offenen Appendektomien nicht signifikant. Die Konzentrationslevel von Isopren, Aceton und Dimethylsulfid zeigten keine signifikanten Unterschiede im Verlauf der beiden Operationsmethoden.

Schlussfolgerung: Trotz der relativ kleinen Stichprobengröße zeigten sich erste Unterschiede in den VOCs der Atemluft zwischen offener und laparoskopischer Appendektomie im Kindes- und Jugendalter. Weitere Studien mit größeren Probandenzahlen sind jedoch nötig, um diese Ergebnisse weiter zu bestätigen.

Abstract

Introduction: Acute appendicitis is one of the most frequent causes of an abdominal emergencies in children and adolescents. Appendectomies can be performed either open or laparoscopically. However, it is still unclear which of the two methods releases less oxidative stress. Analyzation of volatile organic compounds (VOCs) from exhaled breath samples offers the possibility to monitor oxidative stress levels intraoperatively.

Methods: In this prospective randomized pilot study, VOCs in exhaled breath of fourteen children were sampled during an open (n=7) or a laparoscopic (n=7) appendectomy at five different time points (after induction of anesthesia, 15 min, 30 min, 45 min after the first cut and after the last suture). Acetone, isoprene, dimethylsulfide and benzene were chosen as oxidative stress markers and analyzed by gas chromatography and mass spectroscopy at the Technical University Graz.

Results: A significant difference in concentration levels of acetone between the two surgical methods at the first (induction of anesthesia) and second (15 min after the first cut) sampling points was seen. The concentration of dimethylsulfide was significantly different between the two approaches at the first three (after induction of anesthesia, 15 min, 30 min after the first cut) and the last (after the last suture) sampling points. Isoprene and benzene did not show significant concentration changes during the sampling points. There was a significant difference of the benzene level during laparoscopic appendectomies, while it displayed no significant change during open appendectomies. The concentration level of isoprene, acetone and dimethylsulfide showed no significant differences comparing the two methods.

Conclusion: Significant changes of VOC concentrations in breath samples between open and laparoscopic appendectomies were seen despite the small sample size number. Further studies with a larger number of patients are, however, necessary to verify the results.

1 Introduction

A pilot study was performed at the Department of Pediatric and Adolescent Surgery at the Medical University of Graz, Austria, including children suffering from acute appendicitis. The goal was to assess whether open or laparoscopic appendectomy produces more oxidative stress. Breath samples were collected during anesthesia and exhaled volatile organic compounds (VOCs) were analyzed. The procedures were conducted by specialized pediatric surgeons trained in both methods. The planned number of included children was 40, 20 children randomized to the open appendectomy group, the other 20 children to the laparoscopic group. Preliminary results of fourteen patients, seven who underwent laparoscopic surgery and seven who underwent open surgery, were analyzed for this thesis.

The anatomy of the vermiform appendix and the pathophysiology of acute appendicitis will be discussed in Chapter 1.1 to Chapter 1.3 of the present thesis. The diagnosis is thoroughly described in Chapter 1.4. This is followed by a description of various forms of appendicitis in Chapter 1.5. Differential diagnoses are listed in Chapter 1.6. The treatment options and surgical methods will be analyzed in Chapter 1.7. In Chapter 1.8 possible complications are described, followed by a description of post-surgical care in Chapter 1.9. VOCs are the main focus of the thesis and their description is found in Chapter 1.10 and 1.11. Various chemical compounds found in a breath sample are described in Chapter 1.12. Chapter 2 deals with the methods applied in this thesis. Firstly, the enrollment process and randomization process, are explained. This is followed by an illustration of the equipment used and the intra-operative procedure conducted. The statistical background and analysis are described in Chapter 2.4. The results of the pilot study will be presented in Chapter 3, followed by a thorough discussion of the importance of the conducted pilot study and its limitations in Chapter 4 and Chapter 5.

1.1 Anatomy and Physiology of the Vermiform Appendix

The first section of the large intestine is the cecum, which is connected by the ileocecal valve to the last part of the small intestine, the ileum (1). With a length of approximately 8 cm, the appendix is pouch-shaped and its base is connected 1.7 cm below the cecum (2). The wall of the appendix consists of the mucosa, the submucosa, the muscularis propria and the serosa. At the point where the longitudinal muscle fibers of the three taeniae coli meet in the cecum one can find the appendix (3). The vermiform appendix can have various positions: subhepatic, retrocecal, laterocecal or mediocecal posterior to the terminal ileum. Due to the various locations the symptoms of an illness regarding the appendix can be very variable (1). It can usually be found in the lower right quadrant of the abdomen, but it might also be located in the upper left quadrant, the left anterior paramidline or the lower midline (2). The ileocolic artery has a branch called the appendiceal artery that supplies the appendix with blood (1) and the appendicular vein collects the venous blood to the cecal vein, which is drained into the superior mesenteric vein via the ileocolic vein (2). Lymphnodes in the mesoappendix drain the lymph of the cecum and the appendix into the superior mesenteric lymph nodes (2). The appendix is embedded in its own mesentery called the mesoappendix, which is a small part of the mesocolon (1) but it is not as long as the appendix itself, which is the reason for its curled form (2). The appendix is sympathically innervated by the celiac and superior mesenteric ganglia and parasympathically innervated by the vagus nerve (2). Large quantities of lymphoid tissue are found in the mucosa and submucosa of the appendix and similar to the small intestine, germinal centers like the Peyers patches can be identified (4). The appendix is classified as part of the gut-associated lymphoid tissue. Therefore, it has been hypothesized that it is part of the immune system (2). Normal gut flora seems to be stored in the appendix and this might be associated with a faster recovery of the large bowel microbiome in patients following severe gastroenteritis (5).

1.2 Epidemiology, Etiology and Pathophysiology of Appendicitis

Appendicitis represents one of the most frequent surgical emergencies in children and can occur at any age, but the frequency peaks at the age of 10 to 14 years in boys and 15 to 19 years in girls (6, 7). The incidence of perforated appendicitis differs between age groups as well. It is diagnosed in 50% of cases in children younger than 3 years, in 25% of cases in children being aged between 4-6 years and in 10% of cases in school-aged children (8). The main pathological event is thought to be an obstruction of the appendiceal lumen. However, many factors such as lymphoid hyperplasia, fecaliths or foreign bodies might lead to this point. As soon as the lumen is partially or even entirely obstructed, bacteria tend to overgrow. This is accompanied by an increased mucus secretion causing luminal distention and a higher intraluminal pressure. Subsequently, an acute inflammatory response is triggered. The wall becomes ischemic and necrotic accompanied by bacterial translocation. At this point, the stage of a gangrenous appendicitis is reached. An emergency surgical procedure must be performed, otherwise the appendix can perforate, and the contents will spill into the peritoneal cavity. However, if these events occur slowly and the body is able to adapt by controlling the inflammatory process, a localized peritonitis and an appendiceal abscess will form. A diffuse peritonitis will develop if the abscess is not walled off (1). Histologically, appendicitis can be classified into six stages: chronic, acute catarrhal, fibrinous-purulent, phlegmonous, gangrenous and perforated appendicitis (8).

1.3 Bacteriology and Virology of an Appendicitis

The flora of the colon resembles the flora found in the appendix. In a non-inflamed appendix, a variety of anaerobic and aerobic bacteria can be distinguished. In most cases, a positive bacterial culture from the peritoneal cavity can only be obtained in appendicitis cases with higher grade of inflammation. Detectable bacterial growth in a blood culture is likely in 85% of perforated or gangrenous appendicitis patients. If a patient suffers under a persisting infection or maybe a post-surgical infection arises, the results of the culture can be of valuable when the treatment plan is revised (1). In some case, pinworms, *Enterobius vermicularis*, are

found in samples of an acute appendicitis. It is not clear whether they can actually cause the illness, but sometimes they could be the reason for the acute appendicitis and sometimes they only mimic the symptoms of the illness (5).

1.4 Diagnosis of an Appendicitis

The diagnosis of an acute appendicitis is based on the patient's history, physical examination, imaging techniques, such as ultrasound or computer tomography examinations and lab tests. In many cases, patients describe a typical development of abdominal pain, first appearing as generalized abdominal pain including nausea, vomiting and anorexia, followed by a gradual move to the epigastrium, where it intensifies. The pain finally migrates towards the umbilicus and ceases in the right lower quadrant of the abdomen (1). However, the diagnosis of an acutely inflamed appendix in pediatric patients can be challenging. Variable positions of the appendix make it difficult to generalize symptoms. Children might feel the pain radiating to the back, if the appendix is located retrocecal, while diarrhea is usually present, if the appendix has a pelvic position (6). Most patients will usually notice their body´s temperature rise to around 38°C, but this might even be higher if perforation occurs. The physical examination follows a thorough anamnesis. By auscultation and palpation, reduced bowel sounds, tenderness and muscle spasm in the area of the appendix can be found. The spasm will get worse as the inflammation continues. The tenderness is palpable in the right lower abdomen but if the appendix perforates, it can be present in other abdominal quadrants as well. The appendix does not have a fixed position but usually lies around the McBurney's point, where symptoms are usually most severe. A variety of clinical signs to provoke a painful reaction to confirm an appendicitis such as the Rovsing sign, the Psoas sign or the Blumberg sign have been described (1). The Rovsing sign can be triggered by squeezing the left colon and stroking towards the cecum. The Blumberg sign is positive, when pain is triggered by applying pressure on the left lower quadrant of the abdomen and then suddenly releasing it. The Psoas sign is positive, if pain is felt when flexing the right hip joint (4). Several clinical scores, as seen in Table 1, have been developed to support the diagnosis of an acute appendicitis. The Alvarado Score, for example, is seen as a useful tool to

predict an acute appendicitis. If a child receives a score lower than four, an appendicitis is unlikely. A score between five to seven means that an appendicitis is probable. A score of more than eight indicates that an appendicitis is highly possible (9). This score has shown to lower the rates of negative appendectomies and shortens the time until a therapy is started (10). The pediatric appendicitis score (PAS) is similar to the Alvarado scoring system. However, the scores are differently interpreted. A PAS of 0-3 concludes that there is a low risk of an appendicitis. An intermediate risk is found at a score of 4-6 points. An appendicitis is highly probable at a score of 7-10 points. A study concluded that in case of a PAS score of < or = 2 only 2.4% of the patients suffer from an acute appendicitis and when a child presented with a PAS score of > or = 7 only 4% of cases did not show an appendicitis during surgery (11).

	Alvarado Score (9)	**PAS (11)**
Pain migration	1	1
Anorexia	1	1
Nausea/Vomiting	1	1
Tenderness in right lower quadrant	2	2
Rebound pain	1	not included
Temperature elevation	> 37.5 °C: 1	> 38 °C: 1
Leukocytosis	2	1
Left shift of white blood cell count	1	1
Coughing/hopping/percussion	not included	2

Table 1: Alvarado Score versus Pediatric Appendicitis Score

Ultrasound has proven itself the most effective form of imaging techniques for diagnosing an acute appendicitis. It has a sensitivity of 80-100% and a specificity of 78-98%. Using ultrasound is cheap, fast and free of radiation. Its application, however, might be a problem in obese adolescents and heavily depends on the examiner´s knowledge and practice. The diagnosis by ultrasound examination of an

appendicitis can be suspected, if the appendix is enlarged, incompressible and has a diameter greater than 6 mm. It is although difficult to assess if the appendix has ruptured already (6). It is recommended to examine the whole abdomen and the retroperitoneum by ultrasound in order to eliminate possible differential diagnoses. The right hemicolon is identified for orientation. In order to differentiate between the small intestine and the large intestine the haustration of the colon is used as a distinguishing marker. The cecum can be identified as the most caudal part of the right hemicolon (3).

Figure 1 shows an example of an inflamed appendix with a diameter of 8 mm. The patient underwent an open appendectomy and the pathohistological results showed an acute phlegmonous and focally ulcero-phlegmonous appendicitis, accompanied by a fibrinous periappendicitis and mesenteric phlegmon.

Figure 1: Appendix of a 12-year-old boy with acute appendicitis, diameter 8 mm, the patient was operated on the same day.

Computer tomography (CT) is not part of the routine examination of pediatric patients with a suspected appendicitis. It can be used in complicated cases or obese patients. It is not recommended in uncomplicated cases due to the high exposure to radiation and its costs. An intraabdominal abscess with the possibility of solely placing a drain in very ill patients can be an indication for the use of CT imaging (8). The sensitivity for this method is 97% and the specificity 100% (6).

Leucocytes in the blood show a high sensitivity but low specificity, when tested for acute appendicitis. The cell count depends on the stage of inflammation, but leucocyte levels might rise over 10,000/l with an increase of immature neutrophils. Twelve hours after the first symptoms, patients show a rise of C-Reactive Protein (CRP). This is difficult to interpret since virtually all inflammatory abdominal diseases would show such an increase. The highest sensitivity can be achieved if a combined increase of the leucocyte count and the CRP is considered. Erythrocytes and leukocytes can be detected in urine sample in 25% of acute appendicitis patients. This would mean that due to a retrocecal location of the appendix the ureter is inflamed as well. Therefore, various anatomical possibilities have to be considered when proposing a diagnosis (12). A normal white blood cell count does not necessarily exclude the possibility of an acute appendicitis (13). A recent study has indicated that the absence of elevated white blood cell count might lead to a higher negative appendectomy rate in children (14).

At a late stage of inflammation, the complication of an appendix rupture can occur. The pain will increase and spread throughout the abdomen. The heart rate increases as well, as will the temperature spike to around 40°C. The patients will present with an acute abdomen at this point. A “pain-free” interval might be described simultaneously to the appendix rupturing since the tension will be released but pain will increase again (1).

1.5 Various Forms of Appendicitis

1.5.1 Atypical Appendicitis

There are various possibilities why an acutely inflamed appendix might be considered atypical. The position inside the peritoneal cavity or the patient's age might change the symptoms and clinical presentation of the patient (12).

1.5.2 Abnormal Position of the Appendix

A patient will feel less pain if the appendix lies retrocecal, retroileal or subhepatical, since it is not in contact with the parietal peritoneum or the psoas muscle. Therefore, the pain migration from the epigastrium to the lower right abdomen is not present. The ureter might be irritated, leading to a higher urge to urinate. Symptoms might vary as well if the appendix lies in the pelvis. The pain will be noticed in the epigastrium but will present on the left side after moving to the lower abdomen. Symptoms like urination pain and diarrhea might be present (12).

1.5.3 Appendicitis in Children under 5 Years

Toddlers are not able to locate their abdominal pain exactly and have difficulties communicating their symptoms, which makes diagnosing an appendicitis difficult. Their age complicates the diagnosis additionally because many infectious diseases can be the underlying reason for the abdominal pain. There are, however, certain characteristics that might help. The right hip will be held in a flexed position when the child lies on its back. The reflex of the abdominal wall in the lower right quadrant is decreased. The child will favor chest breathing to diaphragmatic breathing (12). Since younger children will present with more atypical symptoms like diarrhea, coughing, sore throat, headache or even earache, it is of great importance to consider acute appendicitis in these cases as a possible diagnosis, in order to reduce morbidity (6). A study has shown that the main symptoms of children under three years, suffering from an acute appendicitis include vomiting, fever, pain, anorexia and diarrhea (15). The diagnosis of an acute neonatal appendicitis is rare,

but its severity should not be underestimated. The incidence lies between 0.04% and 0.2%. The symptoms are difficult to assess due to the fact that they might be similar to necrotizing enterocolitis, gastrointestinal perforation or sepsis (16). The rate of perforation is higher as well as the progression to a peritonitis (17). This increases the mortality rate rapidly to around 25% (16). Diagnosis and the correct therapy take longer and the rate of additional pathological diseases is higher (17), including Morbus Hirschsprung, inguinal or umbilical hernia, meconium ileus, cystic fibrosis or a complication of a necrotizing enterocolitis (16). The child might present with fever, vomiting, non-consistent temperature measurements, lethargy, apnea, food intolerance, discoloration in the right lower abdomen and abdominal pain, as well as bloody diarrhea. The laboratory work-up may show elevated infectious disease parameters. Free fluid as well as an abscess could possibly be detected in an ultrasound examination. Physicians should include an acute appendicitis as a possible differential diagnosis, if risk factors of a necrotizing enterocolitis are missing (16). The fact that the infantile appendix is usually not fixed in a certain position increases the possibility of a diffuse spillage due to a perforation. This occurs more often than in older children, where localized abscesses are formed. Consequently, the symptoms of infants are variable due to this unknown position of the appendix. If the appendix has a retrocecal or sub-serosal position, flank pain or back pain might occur, and rates of perforations are increased (18). Physicians must also think of the possibility of an intestinal malrotation, which might lead to an atypical position of the appendix (16).

1.5.4 Appendicitis in Patients with Crohn’s Disease

Studies suggest that an appendectomy is recommended if an acute appendicitis is suspected in patients suffering from Crohn´s disease. An enterocutaneous fistula may develop in very rare cases (1). Other studies, however, state that if an appendicitis caused by Crohn’s disease develops, the risk of fistula formation spikes (19).

1.5.5 Appendicitis in Children with Cystic Fibrosis

These types of patients usually do not present with an acute appendicitis, but they are more likely to show perforation or an appendix mass. Children are used to the abdominal pain, due to its recurrence because of cystic fibrosis. These children also take medication such as antibiotics and/or steroids more frequently, which might hide the symptoms as well. This all could lead to a delayed diagnosis (5).

1.5.6 Chronic Appendicitis

A patient with chronic appendicitis will present with recurring pain in the lower right quadrant. Adhesions or scar-tissue can be observed (12), as well as prolonged inflammation or fibrosis. Making the correct diagnosis is often difficult. Chronic inflammatory signs can be found in histopathological samples (20). The possible cause of a chronic appendicitis might be a partial obstruction of its lumen (5).
Complications such as intra-abdominal infections, bowel obstruction or perforation might occur (20). Pediatric doctors are often confronted with normal imaging and blood tests (5) but most patients' pain is relieved after an appendectomy (12).

1.6 Differential Diagnoses of Appendicitis

In younger children, a plethora of differential diagnoses has to be considered, since congenital malformations or intussusceptions might cause similar symptoms. Possible differential diagnoses include abdominal pain with no known origin, gastroenteritis, mesenteric lymphadenitis, Meckel's diverticulitis, primary peritonitis, inflammatory bowel disease, neoplasms, urinary tract infection, testicular torsion in male children, ruptured ovarian cysts and ectopic pregnancies in female children (6). The occurrence of those diagnoses vary by age.

Preschool children will commonly be affected by intussusception if younger than three years, Meckel's diverticulitis if pain occurs around the periumbilical area and acute gastroenteritis. Since patients with gastroenteritis suffer from diarrhea as well as vomiting, the differentiation to acute appendicitis can be difficult. Therefore,

it still happens frequently that an appendicitis is misdiagnosed as a gastroenteritis due to the similar symptoms.

Gastroenteritis is also a common differential diagnosis in school-aged children. At this age, omental infarctions occur as well but patients would then present with a palpable mass and a clear pain location.

Adolescents can suffer from inflammatory bowel diseases such as Crohn´s disease or ulcerative colitis. Boys can present with epididymitis or testicular torsion. Adolescent girls can present with differential diagnoses like urinary tract infections, ovarian pathologies like ovarian cysts or a pelvic inflammatory disease (1). Torsion of an ovarian cyst or a tumor might also explain abdominal symptoms. During or after puberty, physicians must also think about a possible ectopic pregnancy or tubal infection that might cause the pain in girls and women.

Chest infection, in form of a basal pneumonia can also be a rare differential diagnosis (5).

1.7 Treatment of Acute Appendicitis

Children with an acute and non-perforated appendicitis should undergo appendectomy. This is still considered the gold standard for children, since the inflammation tends to reoccur. The risk of an imminent sepsis is evaluated, and patients will receive fluids before surgery due to possible dehydration after vomiting (6). A single dose of antibiotics should be given before the appendectomy is conducted as well. A negative appendectomy is more common in women, due to the similar pain characteristics of gynecological diseases (1).

There is number of recently published studies indicating a possible treatment with antibiotics only, in both adults and children. A study published in 2012, for example, has analyzed the outcome of 558 adult patients with acute appendicitis. 79% of those were given antibiotics as a first line treatment for acute appendicitis. 77% responded well to this form of therapy and 23% had to undergo an

appendectomy due to failed improvement under antibiotic treatment. The study concluded that antibiotics can be used as a possible treatment for an acute appendicitis. However, there is an unknown percentage of a long-term risk of relapse, but this must be put in perspective to the risk of complications after a surgical intervention (21). A meta-analysis has revealed that antibiotic treatment of an acute appendicitis in children might be effective as well. The results show no elevated risk of complications, except in cases with appendicoliths. In this case, the appendectomy is still the recommended pathway (22). A Swedish pediatric randomized controlled study generated similar results. 92% of patients were free of symptoms after being treated with antibiotics only. In the follow-up period of one year, appendicitis reoccurred in only 5% of the cases. This was a pilot study of only 24 patients, but the results confirmed other reports that non-operative treatment might be a possible treatment choice (23). A study published in 2016 has included the treatment choice of the families. Again, antibiotics were effective, and morbidity and costs decreased compared to the surgical approach. 75.7% of pediatric patients, who's family decided for a non-surgical treatment, did not have their appendix removed in the one-year follow-up period. These results show less disability days and lower costs during the first year after treatment (24). A recent study evaluating long-term results of antibiotic treatment in case of an acute appendicitis in adults followed 257 patients five years after antibiotic treatment. The results show that 39.1% of patients had an appendectomy after a five year follow up period. This trial supports the possibility of antibiotic treatment regarding long-term outcomes. The results also show a higher overall complication rate, as well as a higher number of sick days after surgical treatment but not difference in the days spent in the hospital (25).

1.7.1 Open Appendectomy

The surgical procedure starts with a transverse skin crease incision in the right lower quadrant above the McBurney point (8). A 3-4cm incision is long enough for a slim child. If needed, the incision can be extended laterally to the flank. A larger skin incision will make more room to operate and the surgery can be done faster but it also means a longer and more painful post-surgical recovery at the hospital. After the skin incision and the division of the subcutaneous tissue, the external oblique muscle is split in the direction of the fibers. This is done by sharp dissection while the splitting of the internal oblique and the transverse muscle is then done by blunt-tipped scissors or even the fingers of the surgeon. After preparation of the transversalis fascia and the preperitoneal fat, the peritoneum is opened with a scalpel or monopolar cautery. The cecum will then be moved into the region of the wound to identify the appendix itself (19). The mesoappendix is dissected and the appendiceal base held by a clamp can be ligated (6). The appendix is sharply removed, and the stump is inverted by an absorbable Z-stitch and/or a purse-string suture. The cecum is replaced after inspection for hemostasis and the pelvis and right paracolic space have to be suctioned. It is not necessary to irrigate the wound if the appendectomy is uncomplicated but in cases of a perforation, the wound is irrigated to remove all visible pus (19). The layers of the abdominal wall are closed separately, and a subcuticular absorbable suture is used for the skin, even in cases of perforation (6). A drainage is only necessary if the appendix stump was not sutured up well due to cecal-phlegmons or if the appendix could not be removed properly (8).

1.7.2 Laparoscopic Appendectomy

Children have a few different anatomical aspects compared to adults, which should be considered when performing laparoscopic surgery. The liver is larger and reaches below the costal margin. The bladder has a more cranial position and should therefore be emptied before surgery and the additional existence of an omphalenteric remnant or an urachus is possible. The abdominal wall of an adult is cylindrically shaped, whereas the younger the child is, the more spherical the wall is shaped. Due to this anatomical difference, the surgeon has more room at a lower pressure in a child's abdominal cavity. If bleeding occurs during a laparoscopic intervention, the surgeon is able to directly press on the vessel and can coagulate or clip it after visualization. If problems appear it is still possible to switch to open abdominal surgery (26).

Indications for a laparoscopic appendectomy include an acute appendicitis, a perforated appendicitis, an appendicitis with coprolith and a retrocecal appendicitis (27). General contraindications for a laparoscopic appendectomy are cardiopulmonary pathologies, pregnancy after the 24th week and acute regional enteritis of the terminal ileum. Specific contraindications to perform a laparoscopic appendectomy are a ruptured proximal appendix, an appendicitis with necrosis or phlegmon of the cecum wall, a carcinoid tumor of the appendix base and an appendiceal carcinoma (28).

Laparoscopic appendectomy is regarded as a safe alternative to the open procedure, but the application varies drastically throughout clinics, even though it is seen just as effective as an open appendectomy. The advantages lie in the shorter hospital stay, lower risk of infection and lower chances of gastrointestinal complications. Disadvantages are that the laparoscopic procedure takes longer and costs more (6). Certain risks are accompanied by this procedure such as difficulties with suturing the stump up, injuring the right ureter, bleeding from the common iliac artery, the epigastric blood vessels or the appendicular artery, that cannot be brought under control, thermal damage to the adjacent tissue like the small intestine or the colon due to the use of electrocautery and difficulties in locating the appendix if it lies retrocecally (28).

A laparoscopic appendectomy can be performed using the extra-abdominal-technique, the mixed-in-out-technique or the intra-abdominal-technique. A single dose of antibiotics is injected when the anesthesia is administrated. A urinary catheter is inserted if a peritonitis and pelvic abscess is present and left until after the post-surgical period. The child is placed in a supine position with the arms next to the body (26). The procedure starts by holding the umbilicus with a strong grip by forceps and lifting it up. The first incision is done by cutting the umbilicus with a scalpel, followed by cutting through the fascia and the peritoneum in order to insert a 10 mm trocar for the optical instruments (8, 26). A curved incision is also possible but only done if a child is not too overweight. The body is tilted into a Trendelenburg position and to the left after the trocar is inserted, in order for the right colon and intestinal loops to move to the left side due to gravity. A blunt trocar with a cannula will be used to inflate the abdominal cavity. The surgeon can avoid CO_2 leakage by administrating a purse string suture alongside the cannula and tying it to the hub. This makes it also possible to move the trocar slightly if more working space is required (26). The insufflation of the peritoneum can either be performed open, by opening the abdominal cavity and placing the trocars under complete view or closed, by inserting a Veress needle underneath the umbilicus. This procedure is not done under vision, therefore small bowl, bladder or vessel injuries may occur. The open procedure is recommended in pediatric centers (27). The pressure plateau of the pneumoperitoneum is chosen depending on the child's size, age and weight. It is usually around 6-8 mm Hg (28). Thereafter, two or three trocars are placed in the abdominal cavity under telescopic control. This is done by adding two 5 mm incisions next to the midline underneath the umbilicus. A third additional incision would be in the right lower quadrant. The appendix is then mobilized and the mesoappendix divided. Two methods can be used to ligate the appendiceal stump, either by endoscopic stapler or endoloops (6). A stapler can be helpful if necrosis of the appendix base occurs. It is also recommended to grab the mesoappendix or the appendix base in the event of an imminent rupture. Surgeons must work very carefully if the appendix might perforate, because manipulation could lead to rupture and peritoneal contamination. It might occur that the appendix shows no sign of infection or inflammation intraoperatively, but the surgeon must search for a possible origin of the symptoms. The inguinal rings, the liver, the gallbladder, a possible Meckel's diverticulum and internal genital organs in females should be

inspected. A peritoneal fluid sample can be taken if necessary, to eliminate potential differential diagnosis (26).

When closing up, the surgeon can use adhesive skin strips if the port holes are 5 mm or less of size. Herniation may occur if larger holes and the umbilical fascia are not closed properly with absorbable sutures.

The laparoscopic approach gains on importance in children with peritonitis because the recovery period of around 10 days from open abdominal surgery is reduced to approximately 6 days. Contraindications include a localized abscess and a complicated appendicitis due to bowel obstruction with abdominal distension (26).

1.7.3 Various Techniques of Laparoscopic Appendectomies

1.7.3.1 The Extra-Abdominal Technique

It is possible to loosen the appendix from adhesions by forceps and extract it through the umbilicus. The appendix is surgically removed outside the abdominal cavity and the stump is tucked back in. The stump and the ligated appendicular artery are looked at laparoscopically before the surgeon closes the child up again. It is not necessary to fixate the optical trocar in this form of procedure. It is difficult to perform this technique if the appendix lies retrocecal or the patient suffers from peritonitis (26).

1.7.3.2 The Mixed Technique

The mesoappendix is coagulated and the appendix is dissected inside the abdominal cavity. After complete detachment, the appendix is pulled out through either the umbilicus or through an incision in the right iliac fossa. The actual appendectomy is again performed outside the abdominal cavity but before closure the stump is inspected laparoscopically as well. This technique is difficult to perform in obese children (26).

1.7.3.3 The Intra-Abdominal Technique

The entire appendectomy is performed inside the abdominal cavity. The surgeon uses an endobag to extract the appendix, which can either be done through the umbilicus or an incision in the right iliac fossa (26).

1.7.3.4 The Singe-Port-Appendectomy

Indications to perform this method are non-perforated appendicitis, phlegmonous appendicitis, appendicitis with impacted stool and obese patients (10). In this technique, only one 10 mm trocar is inserted through the umbilicus. The optical instrument includes an alligator forceps in order to be able to pull the appendix through the abdominal wall to ligate it (8). The trocar has a 5 mm instrument channel and a view at a 6° angle. The appendix is removed outside of the abdominal cavity, just like in open surgery (27). This leaves only one scar in the umbilicus. The method is usually used in cases of chronic appendicitis and when the appendix is removed during a diagnostic laparoscopy (8). Contraindications include a generalized peritonitis after perforation, a retrocecal position of the appendix and adhesions of a prior surgical procedure. This procedure makes it also difficult to search for the Meckel's diverticulum if needed (27).

1.7.3.5 Additional Techniques

The natural orifice transluminal endoscopy surgery (NOTES) is a technique where orifices of the body, such as the vagina, the stomach or the rectum are used for an endoscopic approach (8).

1.7.4 Comparison of Open Appendectomy versus Laparoscopic Appendectomy

The two surgical methods are often compared to each other. The most common results are summarized in Table 2. Studies have shown that it takes 7.6-18.3 minutes less to perform an open appendectomy. Lower rates on a pain scale on the first day after surgery could be identified following laparoscopic appendectomy. Three out of six meta-analyses have shown that the risk of an abdominal abscess is higher when performing laparoscopic surgery, but it was also shown that wound infections occurred less often using this technique. The rate of mortality does not differ between these two surgical approaches. The hospital stay is shortened by 0.16-1.13 days when the child had a laparoscopic appendectomy (29). Studies have also reported that the return to a general diet, to normal activity and to work is faster in laparoscopic appendectomies. The costs of that procedure are slightly higher but are not considered as statistically significant. There seems to be a higher rate of wound infections using the open approach. The overall risk of complications is higher using the open approach (30). Studies have shown that the laparoscopic approach is favored by patients as well as recommended to them by family members and friends. There is also a higher satisfactory rate concerning the size and appearance of their surgical appendectomy scars (31). The laparoscopic approach of an appendectomy also leads to a reduced need of pain medicine and a lower risk of subsequent adhesive bowel obstruction (5).

	Open appendectomy	Laparoscopic appendectomy
Smaller time frame (29)	x	
Lower pain scale values (29)		x
Higher risk of abdominal abscess formation (30)		x
Higher risk of wound infection (30)	x	
Lower risk of adhesive bowel obstruction (5)		x
Less use of pain medication (5)		x
Shorter hospital stay (29)		x
Faster return to diet/daily activity/work (30)		x
Higher overall risk (30)	x	
Recommended by family (31)		x
Higher satisfactory rate (31)		x

Table 2: Open versus Laparoscopic Appendectomy

1.7.5 Interval Appendectomy

This type of surgery is done in a phase where the appendix is not inflamed. It is an elective surgery, performed when an acute appendicitis is either reoccurring often (1) or after an antibiotic treatment, in case of a perforated appendix. Antibiotics can be used to treat complicated appendicitis with perforation at first, while an interval appendectomy is planed approximately two months later. This treatment method is usually successful in adults and children, but complications such as an abscess or a re-inflammation before the interval appendectomy occurs, might develop (32).

1.8 Complications of an Acute Appendicitis

1.8.1 Perforation

The most common complication is the perforation of the appendix that can either occur occult or open. The incidence of perforation has been described ranging between 10-30%, peaking in children under two years and elderly people. If the perforation is occult, an abscess might form in different locations such as a perityhlitical, ileoinguinal, Douglas, lumbal or subphrenical region. If the perforation is open, it will lead to a diffuse peritonitis. Free abdominal air can be detected radiologically (12). The surgeon will irrigate the peritoneal cavity during surgery until the drainage is clear. Administrating a preoperative dose of antibiotics is considered beneficial in order to decrease morbidity (6).

1.8.2 Septic Thrombophlebitis of the Porto-Mesenteric Veins

This condition, also referred to as pylephlebitis, is a complication, which can occur very rarely in cases of acute appendicitis. Thrombophlebitis can be found in the portal venous system, due to a secondary infection of the appendix region, which is subsequently drained into the portal system. This disease is not only associated with acute appendicitis, but also colonic diverticulitis and cholangitis. Due to the antibiotic treatment of these patients, the condition is not seen very often anymore, but it is still considered a condition with a high mortality rate, because the diagnosis can be delayed due to unspecific symptoms, as well as not having it kept in mind as a possible differential diagnosis. Quick diagnosis and intervention are of pivotal importance (33). The management of this disease in children includes intravenous antibiotics treatment, as well as an anticoagulation up to one year and exploratory laparotomy with an appendectomy (34).

1.8.3 Post-Surgery Complications

Wound infections represent the most common postoperative complications following appendectomies. They can develop in the abdominal cavity or in the subcutaneous wound. The occurrence depends on the patient's age, on the physical condition of the patient and on the wound closure type. The rate of wound infection is around 5%, with the incidence of subcutaneous wound infections being even lower when a laparoscopic approach is used (1). An abscess of the abdominal wall is seen in 10-30% of cases. In 2-5% intraabdominal abscesses form, for example in the pelvis and or as a Douglas-abscess. Adhesions, an early ileus and appendiceal stump insufficiency represent other complications (12).

Surgeons operating on a complicated appendicitis in an advanced stage can choose a delayed primary wound closure (1). Studies although have shown that it does not make a difference in wound infections if a subcuticular wound closure is chosen and prophylactic antibiotics are given (35). In very rare cases, a patient might develop adhesive intestinal obstructions. Few studies have focused on possible infertility in female patients suffering from a perforated appendicitis but were not able to withstand critical reviews (6). A mechanical ileus can occur 1-12 months after the appendectomy in 5% of cases. Children will present with abdominal pain, vomiting, constipation and a distended abdomen. 98% of cases have to have surgery again (8).

1.9 Post-Surgical Care

A single dose of prophylactic antibiotics is used in uncomplicated cases of appendicitis. However, in cases with peritonitis an intravenous broad-spectrum antibiotic therapy should be administered for 4-6 days, according to the inflammatory parameters of the blood. Thereafter, an oral antibiotic therapy can be given for 4-6 days aimed at aerobic and anaerobic bacteria. If a child is under five years of age or a severe peritonitis is present, urinary catheters can be used. The patient can be allowed food intake a couple hours after the surgery but must wait a couple days until initial recovery if he or she suffered from a peritonitis. The drain

for abscesses will be removed after three days. The child will remain in the hospital for around 48 hours in uncomplicated appendectomy cases but must stay 4-6 days if an appendicular abscess or a peritonitis occurred (26). Post-surgical pain has to be evaluated continuously by the Visual Analogue Scale or the KUS-Scale (Kinder Unbehagen und Schmerzskala). The therapy should be aligned with the recommended grade-scheme, starting with Paracetamol, Metamizol or Diclophenac intravenously. If the pain cannot be controlled by these medications, it can be treated with Step-2 analgesics such as Nalbuphin or Piritramid, applied intravenously as well (8).

1.10 Volatile Organic Compounds in regard to Oxidative Stress

Oxidative stress is defined as an imbalance in favor of prooxidants to antioxidants, which leads to reactive oxygen species (ROS) accumulation in cells. The oxygen metabolism creates ROS as by-products, mainly by mitochondria. As long as the system is not exhausted, it is possible to detoxify the surplus. An excess leads to a depletion of antioxidants and a higher presence of ROS. Free radicals are created from ROS through enzymatic reaction, like oxidase enzymes for example, or through non-enzymatic reactions, due to organic compounds being able to interact with oxygen. This process can either happen endogenously, in case of inflammation, infection etc. or exogenously, through environmental exposure to heavy metals or pollutants. These free radicals are beneficial for the body as long as the concentration is kept low (36). The immune system needs them to eradicate pathological microorganisms and ROS serve as cellular signals in regulatory processes (37). Oxidative stress and subsequently cell damage, is present at the point when the concentration of free radicals rises (36). The protection of cells by antioxidants, such as glutathione, superoxide dismutase, catalase and vitamin C and E, can get exhausted and even attacked, through ischemia or reperfusion (38). The body constantly produces ROS and free radicals, leading to oxidative stress, due to aerobic and defensive mechanisms, radiation and exogenic factors in the air or in food, but the importance lies in the balance of the concentration of anti- and prooxidants, so exhaustion can be avoided (37). Reactive oxygen species that are not kept under control, are to blame for ischemic induced organ dysfunction or organ

failure. This is of concern, because these pathologies are reasons for morbidity and mortality after major abdominal and cardiovascular surgeries (38). There is, at the moment, no single biomarker that can measure oxidative stress. Since blood and tissue biomarkers concerning oxidative stress vary as well, it is important to differentiate between these two (39).

1.11 Oxidative Stress Production during an Appendectomy

During an appendicitis, oxygen is taken up at a higher level causing a respiratory burst and free radicals. Subsequently, a higher rate of lipid peroxidation due to the increase of free radicals, leads to a higher microvascular permeability, intestinal edema and inflammatory cell infiltration and activation. This causes higher production of arachidonic acid, cytokines and chemokines. Additionally, mast cells and polymorphonuclear leukocytes are found in the inflamed appendix. The result is a larger production of free radicals, finally leading to cell death (40).

Oxidative stress is generated in laparoscopic procedures due to inflation-deflation related splanchnic ischemia-reperfusion, hemodynamic changes and CO_2 pneumoperitoneum. In open surgery, the peritoneal cavity is exposed to room air and therefore a higher oxygen concentration. The bowel is manipulated considerably more, which also leads to a higher level of oxidative stress. A study published in 2016 has shown that the level of the total oxidant status and the oxidative stress index, 24 hours postoperatively, are much higher in patients who underwent open appendectomy compared to laparoscopic appendectomy. The C-reactive protein (CRP) is lower in patients who underwent laparoscopic appendectomy. The total antioxidant status showed no difference in the two forms of appendectomy. The study stated that the CRP reduction 24 hours post-surgery, correlated with the total oxidant status in laparoscopic surgery (39).

The measurement of oxidative stress during medical procedures has found wide interest. A study from 2016 has focused on a new oxidative stress marker in blood samples that could help diagnose an appendicitis. They examined the balance of thiol and disulphide and noticed a shift towards the disulphide

concentration when an appendicitis was present (41). A study from the year 2015 measured the total oxidant status and the antioxidant status in blood samples of patients suffering from an acute appendicitis. These concentrations were compared to the oxidant level of a control group. The oxidative stress status was higher in those with a diagnosis of an appendicitis. The study concluded that this is due to the inflammation progression (42). Another trial measured the total antioxidant capacity in children showing signs of an appendicitis compared to healthy ones. The results showed that the antioxidative stress response of patients suffering from an acute appendicitis is higher than in those with a non-surgical illness and that the children do not show signs of an antioxidant capacity deficiency in blood plasma samples (43).

1.12 Breath Analysis

Breath consists of a mixture of nitrogen, oxygen, carbon dioxide, water and inert gases as well as volatile organic compounds that are in the nmol/l-pmol/l range. Nitric oxid, carbon monoxide, ethane, pentane, acetone, isoprene, isoprostane and peroxynitrite are considered endogenous compounds of the human breath. Usually, nonvolatile substances can be detected in breath analyses as well, including isoprostane, cytokines, leukotriene and hydrogen peroxide (40). In healthy individuals, more than 800 volatile organic compounds have been identified in a breath sample. Many of those are not produced endogenously and can vary due to smoking, food consumption and medication intake (44). A study found numerous components in breath samples such as ketones, like 2-butanone and 2-pentanone, aldehydes such as acetaldyde and benzaldehyde, alcohols like 1-propanol and 2-propanol, as well as methanol and ethanol, acids and esters such as acetic acid and propionic acid, furans, aromatics hydrocarbons, saturated hydrocarbons, like propane, n-butane and n-pentane, unsaturated hydrocarbons such as isoprene, sulfur compounds, like dimethylsulfide, allyl methyl sulfide and methyl propyl sulfide and compounds that include nitrogen such as acetonitrile, N,N-diethylformamide and N,N-dimethylformamide (45).

A correlation between the clinical condition of a patient and the exhalation of VOCs can be detected. A number of VOCs are released in the exhaled breath only a few minutes after an increase of the body´s oxidative stress level and they can therefore be used to analyze potential pathological changes (38).

The air in the room where the breath sample is taken can, however, alter the results. A study has shown higher levels of propanal, decane, 2-butanone, benzaldehyde, hexanal, 3-methyl thiophene, 2-methyl butane, ethylacetate, acetonitrile and 2-methyl hexane in the room air sample when compared to patients´ breath samples. The authors concluded that these are possible exogenous compounds but can still be found in breath samples. The same study also found that unsaturated hydrocarbons such as 1,3-butadiene, furans such as 2-methyl furan and acetonitrile were more often detected in smoke related samples (46). A different study also has revealed that higher levels of furans are found in a smoker's breath, compared to non-smokers. This again points to furans possibly being exogenous compounds in breath samples. Higher levels of aromatic hydrocarbons are also found in breath samples taken from a group of smokers compared to a non-smoking group (45).

This study will focus on the volatile organic compounds isoprene, acetone, benzene and dimethylsulfide.

1.12.1 Isoprene

The unsaturated hydrocarbon 2-methylbutadiene-1,3, isoprene, can be used as a breath marker for oxidative damage in the body. It is formed in the mevalonic pathway of cholesterol synthesis. Isoprene can be found in the periphery of the body (44) and is always detected in a human´s breath sample (40). This marker has to be interpreted with caution since it shows a circadian rhythm with higher levels in the morning and lower levels in the late afternoon. Children exhale a much lower amount of isoprene, so it is concluded, that it its levels are age dependent (40). A study found that a higher level of isoprene exhalation might be related to oxidative stress, due to the fact that the breath isoprene concentration was elevated in acute

myocardial infarction patients in comparison to patients without symptoms and patients with stable angina pectoris (47).

1.12.2 Acetone

The propylketone, acetone, is found in a large amount in a human´s breath sample. Acetone is produced through acetoacetate-decarboxylase from acetoacetate (44) by hepatocytes if there is an excess of Acetyl-CoA, derived from lipid peroxidation (40). These are the last steps of the ketone-body pathway, which is necessary for secondary energy supply (44). A study analyzing exogenous factors in breath samples, has found acetone to be detected in every VOC sample taken, including breath and urine samples. The authors have also shown a higher presence of acetic acid and propionic acid in their breath samples in comparison to their room air samples, which points to an endogenous compound (45). Acetone is oxidized, like other ketones, in the peripheral tissue and increases if the patient is fasting. This can be measured in a blood sample. Acetone levels in the exhaled air are higher, if the patient suffers from uncontrolled diabetes mellitus. Ethanol, 2-propanol, is reduced from acetone through enzymes, but its concentration is always lower than the acetone concentration in a breath sample. Ethanol concentrations in a breath sample can be derived from the intestinal bacterial flora (40).

In a recently published study, volatile organic compounds such as pentane, acetone and isoprene were measured during cardiac surgery in order to assess the effects of the surgery and the ischemic situation, as well as the reperfusion in regard to the dextrose and cholesterol metabolism, metabolic stress and lipolysis. The acetone level positively correlated with the serum C-reactive protein. Isoprene was exhaled in higher amounts in patients with a high cardiac index. The study concluded that volatile organic compounds correlate with the clinical condition of the patients, in regard to acetone, which reflects metabolic stress and pentane, which was elevated during surgery and also isoprene, which correlated with the cardiac output. The study described that the level of exhaled pentane rose after the sternotomy, and the authors assumed that this is because of oxidative stress, caused by the saw and electrocautery (38).

1.12.3 Benzene

In the present study, benzene was identified in the volatile organic compound samples. It is an aromatic hydrocarbon in a liquid state with a gasoline-like smell. Benzene is an intermediate chemical substance that can also be applied as a solvent. Exposure to the body causes neurological damages, bone marrow interferences and immune system failures. It is a human carcinogen und can be linked to a higher risk of lymphatic cancer, as well as hematopoietic cancer. It also increases the chance of developing an acute myelogenous leukemia and a chronic lymphocytic leukemia. Usually found in industries, benzene can also occur naturally. It can be found after forest fires and in volcanoes. Benzene is a natural component of gasoline, as well as cigarette smoke. Usually exposure to this chemical happens on a daily basis, by inhaling air, which is contaminated at the gas station or from cigarette smoke. Benzene can also be found in glues, detergents and even paints, and might contaminate the living environment through these products (48). A trial evaluated the benzene concentration in blood samples from non-smokers and smokers. Benzene was detected in every sample, which concludes that there is a ubiquitous exposure in our environment. Smokers although did show higher levels of benzene in their blood samples, while non-smokers had the same levels of benzene in their body, as measured in outdoor and indoor air samples (49). Children under one year of age, whose parents smoked at home, showed higher levels of benzene compared to children whose parents did not expose them to cigarette smoke. The heating type used in a home is also at fault of higher benzene values in blood samples. Higher levels are seen when coal and wood heating systems are used instead of a central heating system (50). Generally, a higher exposure to benzene has a considerable impact on a child´s respiratory health. Children have a tendency to develop diseases such as asthma, pulmonary infections and a decline of lung function (51).

1.12.4 Dimethylsulfide

Dimethylsulfide, an alkanethiol, is a degradation product of methanethiol. Methanethiol is considered a human metabolite, but it is also possible to absorb it through skin contact and is highly toxic when inhaled. It is a substance that can be found in various tissue samples of humans and the chemical compound is considered at fault for the smell of bad breath (50), due to anaerobic bacteria inside the mouth cavity (52). Dimethylsulfide, a chemical found in the blood and organs of humans, is created through bacterial degradation of proteins and methanthiol (53). It is highly toxic and attacks the central nervous system at high concentrations. Certain foods also contain an amount of methanethiol, such as cheese and a variety of nuts (50). A pilot study from Taiwan published results from VOC concentrations before and after a surgery in operating room personnel. The concentration of dimethylsulfide was significantly higher after a surgery was conducted. Therefore, questions about the chemical exposure during a surgery were posed (54).

1.13 Aim of the Study

The present study aimed to detect changes of oxidative stress markers in breath samples from patients aged between 6-18 years undergoing either an open or a laparoscopic appendectomy due to an acute appendicitis. The difference between oxidative stress levels during those two surgery methods has not been elucidated yet. Changes in oxidative stress concentrations can lead to a better understanding of the two appendectomy approaches and a better surgical setting, where suggestions regarding pre- or intraoperative anti-oxidative treatments, are included in the surgical protocol. Trial results could also be an impulse to incorporate new or additional physical examinations in the daily surgical treatment plan.

2 Methods

2.1 Recruitment and Enrollment

Following approval by the local ethical committee (EK 30-120 ex17/18), all children presenting to the Department of Pediatric and Adolescent Surgery of the Medical University of Graz with acute appendicitis were asked to participate in the study. If they were interested, a consent form was handed out and had to be signed by the patients and/or care takers. Children between 6 and 18 years of age, with an acute non-perforated appendicitis were included in the trial. By using the Alvarado Score, the pediatric appendicitis score, clinical symptoms, imaging techniques and laboratory markers, the diagnosis of an acute appendicitis was made. Exclusion criteria included an autoimmune disease, or any other diagnosed chronic illness. A child was also not a fit for the study if it presented with an additional infection to the acute appendicitis, suffered from a systemic inflammatory response syndrome, had a reduced liver function, or took medication affecting the Cytochrom-P450 System, also including cortisone. If the parents did not give their consent, the child was also not be able to participate. Children diagnosed with an atypical acute appendicitis such as a perforation of the appendix or a formation of an abscess or children whose surgical method was modified intraoperatively from a laparoscopic surgery to an open laparotomy, were also excluded from the study.

2.2 Randomization

The next step after recruitment was to include the patient in the randomization process. Patients who agreed to participate in the trial were randomized by a web-based program called “Randomizer for Clinical Trials”, found on www.randomizer.at, at the Department of Medical Informatics, Statistics and Documentation. A 1:1 ratio was chosen. The surgeon was not blinded to the procedure because he or she needed to know pre-operatively, which method would be performed. If a typical acute appendicitis turned into an atypical, or if the surgeon had to convert from the laparoscopic surgical method to an open appendectomy, the patient was removed from the randomization process and the data was not evaluated. Patients data were

pseudonymized with a numeric code, which was used to collect results in an Excel®-Table. This data was only accessed by authorized personnel. Data breach was the only threat in this trial, which was regulated by encoding all patients' data and authorizing only trial relevant personnel to look into the collected results.

2.3 Procedure

A newly developed technology to sample breath air was applied intraoperatively while the patient was being anesthetized. Volatile organic compounds were extracted through a syringe from the exhaled air of the patient and stored in a sample tube for analyzation by gas chromatography and mass spectroscopy at the cooperation partner at the Institute of Analytical Chemistry and Food Chemistry at the Technical University of Graz.

2.3.1 Equipment

Every patient required new or sterilized sampling equipment, in order to extract the exhaled air hygienically and without contaminations. There are a number of medical products necessary for breath analyzation. This includes a T-shaped plastic piece that connects the anesthetic machine with the sampling device, through a three-way valve. A 20ml glass syringe was placed on the second port of the three-way valve, as seen in Figure 2. It was needed to extract the exhaled air and store it temporally, before injecting it into a test tube. A plastic tube connected the third port of the three-way valve with a needle. This needle was used to inject the breath sample into a small test tube, labeled according to the patient and the extraction time. The test tubes were flushed out with nitrogen before they were filled with the patient's breath sample. This increased the level of purity. It was nevertheless necessary to subtract the components of the room air from the breath sample, in order to receive outcomes that are not distorted by the air in the operating rooms. This was administered by including two air room samples per patient, for distinction of the air components.

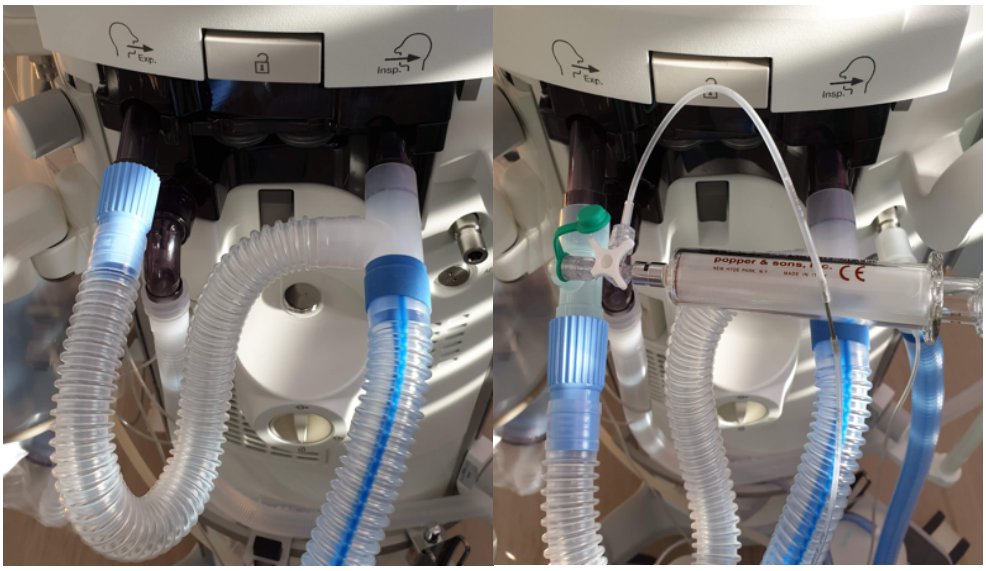


Figure 2: Equipment to sample breath air during anesthesia. The panel on the right shows the glass syringe coupled to the three-way valve

2.3.2 Sampling Method and Anesthesia Protocol

Before the anesthesia started, the equipment of the study had to be put in place. The T-shaped plastic piece was connected to the exhalation tube of the anesthetic machine before use. After this step, the anesthetics could be applied. The medication given to the child prior to the surgery in order to start sedation were fentanyl, piritramide, propofol and rocuronium. The anesthetics used during surgery in order to tamper as little as possible with the results of the breath analysis were propofol, remifentanil and ibuprofen, in order to keep the child sedated and pain free. Fentanyl belongs to the group of synthetic morphine agonists. It shows an analgesic effect after only three to four minutes for around twenty to forty minutes. It is metabolized in the liver und eliminated by the kidneys. Fentanyl is used as the analgesic component in a total intravenous anesthesia with controlled ventilation (55). Piritramide is an opioid and analgesic medication. It also has a sedative component. It is used as a pain killer, especially after surgery. The medication is metabolized in the liver as well and eliminated by the kidneys and through faeces (56). Propofol is used for the induction and maintenance of general anesthesia. It is a short-acting sedative, coming into effect after only approximately thirty second. It lasts for about five minutes. The liver metabolizes the medication and is eliminated by the kidneys. It can lead to vasodilation and respiratory depression, but also has

an anticonvulsive effect and decrease the intracranial pressure (57). Rocuronium is a non-depolarized muscle relaxant that is used during the induction of anesthesia in surgery. Its effect takes place after around 60 seconds and holds on for approximately 20-40 minutes. It has no sedative component and leads to a paralysis of the respiratory muscles, due to its muscle relaxing effect. The medication is not metabolized and eliminated through bile and urine (58). Remifentanil is used during general anesthesia as an analgesic drug. It is short acting and does not accumulate. It is not metabolized by the liver, but by blood and plasma esterases. It has rapid clearance and is usually applied by a continuous infusion. The drug shows effects after only one minute and last for approximately 3-10 minutes. There is no sedative effect, but strong respiratory depressive symptoms. Ibuprofen is given as an analgesic drug at the end surgery, to reduce post-surgical pain. It has an anti-inflammatory, anti-pyretic and analgesic component. Ibuprofen has its peak effect after around one hour, is metabolized by the liver and eliminated by the kidneys (59).

A student or a doctor trained for this procedure collected the breath samples during the necessary time periods. The samples were tagged and stored in a container protected from light. This container was then brought to the Technical University of Graz for analyzation by gas chromatography and mass spectrometry right after surgery. Blood was drawn prior to the surgery but after sedation and a second blood sample was taken after the last suture and before the child woke up after sedation. These blood samples, specifically the level of the C-reactive protein and the white blood cell count, were evaluated by the in-house laboratories.

Two VOC samples were taken intraoperatively at six time points in duplicate as seen in Table 3. This means there were twelve samples in total per patient in the trial.

T1	After induction of anesthesia (n=2)
T2	15 minutes after first incision (n=2)
T3	30 minutes after first incision (n=2)
T4	45 minutes after first incision (n=2)
T5	After the last suture (n=2)
T6	Room air sample (n=2)

Table 3: Time points for VOC sampling

2.3.3 Histological Grading

The extracted appendix vermiformis was sent to the Department of Pathology at the Medical University of Graz. A histopathological classification into the four degrees of severity, acute focal appendicitis, acute suppurative appendicitis, acute gangrenous appendicitis and perforated appendicitis, took place.

2.4 Statistical Analysis

2.4.1 Statistical Background

Statistical tests are based on hypotheses. The null hypotheses in this trial stated that there was no difference of VOC concentrations between the two surgical groups, while the alternative hypotheses would have suggested that there was a difference to be seen. Statistical calculations were implemented to calculate the so-called p-value. This could tell whether there was a significant difference between the two groups. A p-value larger than 0.05, meant no significant difference could be detected, meaning that the null-hypotheses could not be rejected. If the p-value was smaller than 0.05, the alternative hypotheses should have been accepted. The level of significance was stronger the smaller the p-value was, which lead to the

assumption that the null hypotheses could be rejected because of a strong indication that the difference is unquestionable and not due to chance alone (60).

There are a number of factors that might impact the results of a study and these have to be acknowledged before collecting samples. This includes chance, bias and confounding. Bias can also be called a systematic error, while confounding factors can also be named intermediary variables. The latter occurs when a causal relationship between the dependent and independent variable is thought to be found but is actually misleading (61).

The data was collected in Excel® and analyzed by the statistical software SPSS 26.0®. The conducted study was a pilot study and used the internationally accepted significance level α of 5% and a study power β of 80%. There was not enough literature, nor similar studies available to use as a comparison, in order to calculate a legitimate sample size. The goal of the study was therefore to pose as a hypotheses-generating trial and all results had to be assessed as solely explorative. 14 patients were included in the study, with 7 participating in each group. Only strong effects could be detected due to the small sample size.

2.4.2 Analysis of Distribution

Before analyzing data, it was important to define whether the variables follow a normal or non-normal distribution. If the data presented itself as normally distributed, parametric statistical tests like the t-test could have been applied, which show advantages such as a higher power to analyze differences between the two study groups. Tests for parametric data were used in case of continuous variables and a symmetrical form of distribution. The mean showed the central tendency and the standard deviation provided us with information of the dispersion of the data. If the data showed a non-normal distribution, transformation could have been used to change the dependent variable's scale of measurement. This includes a log-transformation, a reciprocal transformation or a square root transformation, which must be considered when interpreting the results of the study, because from the point of interception, all the data must be read on the newly transformed scale. It was although not possible to do this with our collected data. A strong indication for

a normal distribution was similar values of mean and median. If this was not the case, the data was so-called skewed. Skewness is defined as a certain asymmetry of the distribution and can be expressed by numbers. The number zero would indicate a normal distribution. The second value which assesses normal distribution well, is the kurtosis. It tells us about the width of the peak and the weight of the tails. A value close to three indicates a normal distribution. Both values could indicate a wrong result, if the sample size is not large enough. In this study we have used a statistical test, the Kolmogorov-Smirnov test to calculate the distribution of the collected data, but we ultimately assumed that the data cannot be normally distributed, due to the small sample size. There is although no cut-off number in a sample size, which could help to identify which distribution the data follows, which makes the analyses sometimes difficult to assess. Non-parametric tests were used throughout the study to analyze the results. Distribution and variance of the data were not of importance while applying those tests (60).

2.4.3 Mann-Whitney-U Test

In the study we used the Mann-Whitney-U Test to evaluate differences in VOC concentrations at the respective time points between the two independent surgical groups. These calculations did not give information about the changes of concentration over time during the surgery, but could tell, if there was a significant difference between the concentrations of a chosen VOC at a given sampling point, among the two surgery approaches. The Mann-Whitney-U Test, which ranks the data set, was used due to a non-normal distribution (60, 62)

2.4.4 Friedman Test

The Friedman Test was applied to examine differences among multiple sampling points. We analyzed whether the concentration of each individual VOC changes during the course of the surgery. The calculations were done separately for each surgery method. In order to use the analysis of variance (ANOVA) with repeated measures, the data must have been normally distributed and one must have assumed sphericity, by utilizing the Mauchly´s test, which means that the variance of different samples among various time points is roughly similar. It is not

absolutely necessary to rely on sphericity because the statistical program provides correctional values, that can be used, when the condition of sphericity is not met, such as the Greenhouse-Geisser or the Huynh-Feldt estimate. It occurs in small sample sizes that strong violations are not significant, and it happens in large samples sizes that, rather small deviations are seen as significant. These criteria were not met by our data and therefore the Friedman´s ANOVA, which is based on data ranking, was used instead. This statistical form of analyzes can be applied when it is necessary to calculate differences between variables that are dependent, such as concentration changes of a VOC from the start to the end of an appendectomy. A pairwise comparison is used, if significant differences are detected, because the Friedman´s ANOVA does not provide information about the certain time points, at which the concentration changes significantly. The p-values have to be adjusted by the Bonferroni correction, due to the multiple number of tests when using pairwise comparison. This is applied to avoid an increase of the Type I error rate. The Cohen´s d, as seen in Figure 3, assessed the effect strength of changing concentrations among various sampling points. Cohen´s d can also be converted into the correlation coefficient r (62).

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(s_1^2 + s_2^2)/2}}$$

Figure 3: Cohen`s d equation

2.4.5 Kendall-Tau Correlation

This study analyzed correlations between independent variables using the Kendall-Tau statistic. It allowed us to recognize whether the variables can be put into relation to each other. Kendall-Tau correlation testings can be applied when the data is not normally distributed and it is favored over other forms of non-parametric correlation analyzations because it is also useful in small sample sizes, such as ours was. By applying Kendall-Tau statistics, it is possible to draw accurate generalizations in a population. The resulting coefficients have values between -1, indicating a negative relation, to +1, indicating a positive relation. The value of 0

would mean that a linear relationship is not present (62). Our sample size of 14 patients was rather small and therefore correlations were found among all variables but with no significance. It was therefore not possible to draw conclusions about a population.

3 Results

3.1 General Results

A total of fourteen patients were included in the preliminary results for this thesis. These fourteen patients consisted of eight males and six females. The median ($\tilde{x}$) age was 11.5 years with an interquartile range (IQR) of 4. The Pediatric Appendicitis Score showed a $\tilde{x}$ = 7 with an IQR = 5 and the Alvarado Score a $\tilde{x}$ = 7 with an IQR = 4. The pre-operative blood samples showed a $\tilde{x}$ = 13.95 mg/dl and an IQR = 36.8 for the CRP and a $\tilde{x}$ = 11.87 G/l and an IQR = 8.22 for the white blood cell count. The mean diameter of the appendix in the ultrasound was at $\tilde{x}$ = 8.5 mm with an IQR = 3.3.

The patients were randomized to seven open appendectomies and seven laparoscopic appendectomies. The appendix was evaluated histopathologically and four samples were graded as acute focal appendicitis and ten as acute gangrenous appendicitis.

Table 4 shows a comparison of the collected data from the two surgical methods in this pilot study. The values are presented as median and inter-quartile range due to the sample size of seven per group.

Characteristics	Open Appendectomy (n=7)	Laparoscopic Appendectomy (n=7)	p-value
Age (years)	11 (6)	12 (4)	0.805
Gender (m/f)	5/2	3/4	0.280
Surgery duration (minutes)	57 (48)	63 (13)	0.383
C-Reactive Protein pre-operatively (mg/dl)	15 (45)	5.9 (36.8)	0.259
White Blood Cell Count (10^9/l)	11.34 (7.95)	12.4 (10.04)	0.805
PAS	6 (5)	7 (4)	0.535
Alvarado Score	7 (4)	8 (4)	0.456
Appendix Diameter in Sonography (mm)	7.5 (4)	9 (4)	0.165
Appendizitis grading	3 (2)	3 (2)	1.00

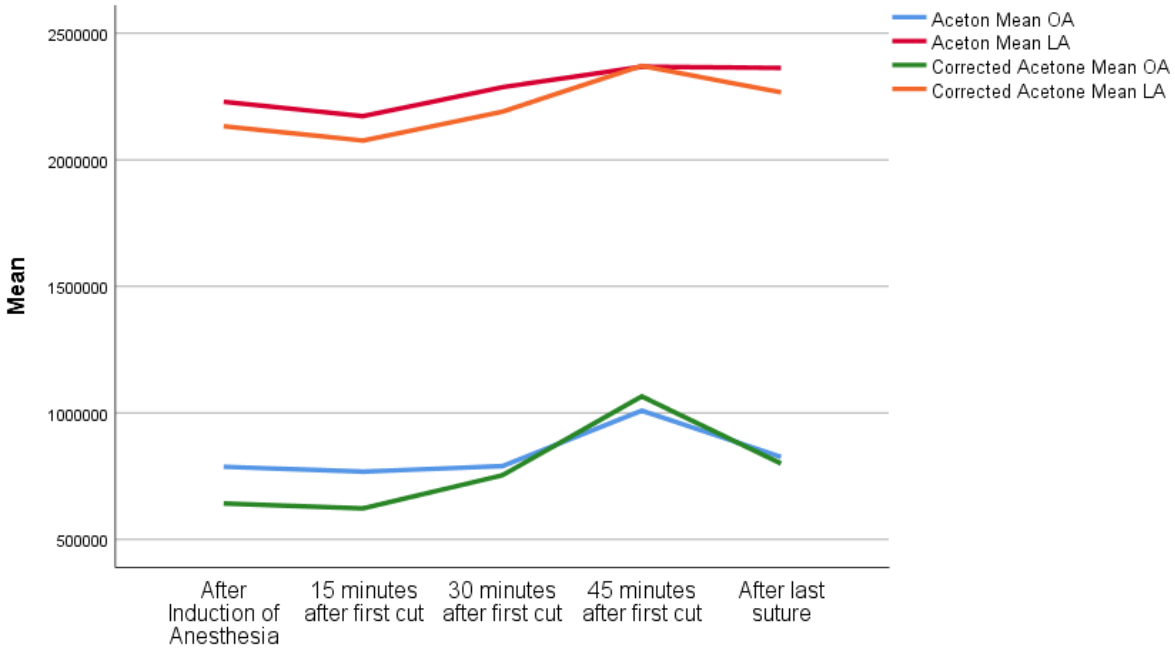
Table 4: Group Statistics with Median and Inter-Quartile-Range

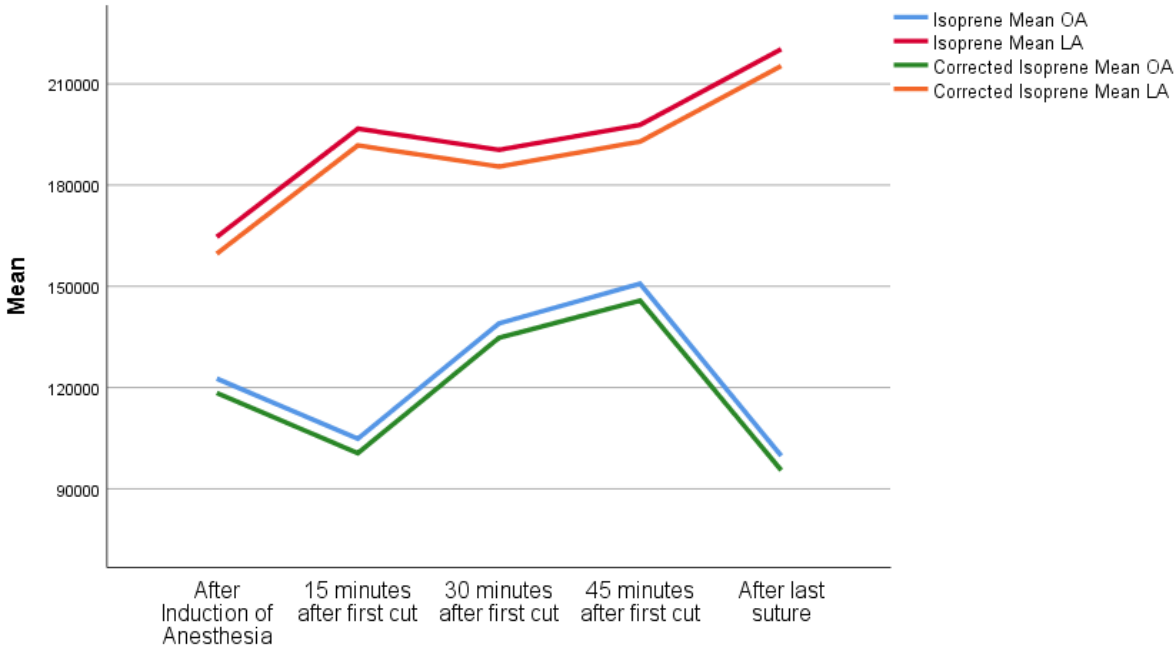
3.2 Missing Values VOC Analysis

There were some values missing, due to the fact that not all surgeries took the required time for sampling. In two cases, the fourth sample, at the time point of 45 minutes into surgery, could not be taken. The dimethylsulfide room samples were not included twice. There were no values of the chemical benzene found once in the room sample. All these missing cases were considered during the calculations.

3.3 Adjusted VOC Concentration Levels

Two breath samples were taken at each time point and the mean value of acetone, dimethylsulfide, benzene and isoprene concentrations were calculated. Room samples were taken in order to be able to subtract the VOC levels of the room air from the breath sample. Figure 4 shows the consequence of this subtraction during the two forms of surgery. Each graph represents one VOC, acetone, isoprene, dimethylsulfide and benzene and the two surgery methods, to show a visual comparison.


Aceton Mean OA
Aceton Mean LA
Corrected Acetone Mean OA
Corrected Acetone Mean LA
Mean
2500000
2000000
1500000
1000000
500000
After Induction of Anesthesia
15 minutes after first cut
30 minutes after first cut
45 minutes after first cut
After last suture


Isoprene Mean OA
Isoprene Mean LA
Corrected Isoprene Mean OA
Corrected Isoprene Mean LA
Mean
210000
180000
150000
120000
90000
After Induction of Anesthesia
15 minutes after first cut
30 minutes after first cut
45 minutes after first cut
After last suture

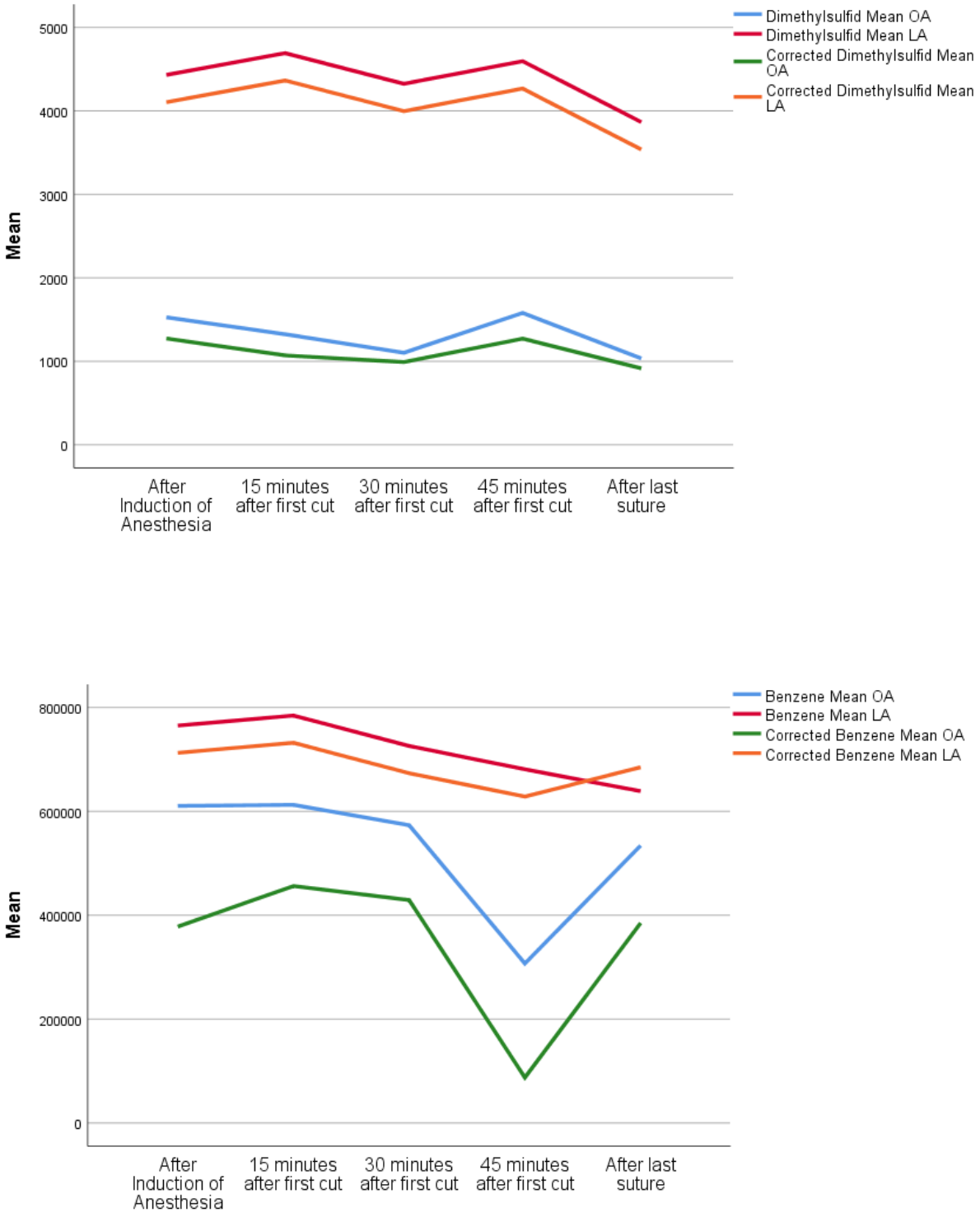


Figure 4: Line graphs with adjusted means of the four VOCs measured. Effects of the subtraction of the concentration of the respective VOC measured in room air samples from breath air samples

3.4 Acetone

3.4.1 Friedman Test with Repeated Measurements

The change of the acetone concentration during both surgery methods was analyzed separately using the non-parametric Friedman Test. The open and the laparoscopic appendectomies showed no significant acetone concentration change over time with a significance level of $p = 0.63$ and $p = 0.45$, respectively.

3.4.2 Mann-Whitney-U Test

The Mann-Whitney-U Test was applied in order to analyze the concentration difference of acetone during the various sampling points among the two surgery approaches. The test showed significant values of $p = 0.038$ at the first and at the second time point, with a medium effect of $r = 0.9$. No significant concentration differences were found during the third, fourth and fifth sampling time.

3.5 Isoprene

3.5.1 Friedman Test with Repeated Measurements

The open and the laparoscopic appendectomies showed no significant isoprene concentration change over time with a significance level of 0.66 and 0.7 respectively.

3.5.2 Mann-Whitney-U Test

No significant differences of isoprene concentration among the two surgery methods were found during any time point.

3.6 Dimethylsulfide

3.6.1 Friedman Test with Repeated Measurements

The open and the laparoscopic appendectomies showed no significant dimethylsulfide concentration change over time with a significance level of 0.52 and 0.75 respectively.

3.6.2 Mann-Whitney-U Test

A significant difference of dimethylsulfide concentrations could be found among the two appendectomy methods during all sampling points. The significance of the first and second mean adjusted dimethylsulfide samples was at $p = 0.017$, with a medium effect of 0.41. The concentration level at the third and fifth time point had a significance of $p = 0.08$, with a medium effect of 0.42. During the fourth sampling point, the concentration showed a significance of $p = 0.048$, with a medium effect of 0.38.

3.7 Benzene

3.7.1 Friedman Test with Repeated Measurements

The open appendectomies showed no significant change of concentration during the surgeries with a significance level of $p = 0.63$. A significant change of the benzene concentration occurred during the laparoscopic approach with a significance level of 0.00. In order to analyze at what time points the difference took place and with what effect, a pairwise comparison in consideration of the Bonferroni correction was conducted. There was a significant difference between the 1. and 5. time point, with a p value of 0.003 and a strong effect of 1.05. Between the 2. and 5. time point a significant difference with a p value of 0.005 and a strong effect of 1.00, was seen. Between the 1. and 4. time point a significant p value of 0.035 and a strong effect of 0.84 was calculated.

3.7.2 Mann-Whitney-U Test

No significant differences of benzene concentration between the two surgery methods were found during any sampling point.

3.8 Correlation

Due to the very small sample size the Kendall-Tau-b Test was applied to calculate correlations between markers of infection and the concentration of the four VOCs directly after induction of anesthesia. A correlation could be detected in all calculations, as seen in Table 5, but a significance could not be reached regarding the VOC breath samples, possibly due to the small sampling size.

Table 5 Kendall-Tau-b

			C-Reaktives Protein pre operatively	White blood cell Count pre operatively	Pediatric Appendicitis Score	Alvarado Score	Appendicitis grading	Appendix Diameter in Sonography
Kendall-Tau-b	C-Reaktives Protein pre operatively	Correlationscoefficient	1,000	,221	,258	,219	,033	,185
		Sig. (2 tailed)	.	,273	,219	,292	,897	,374
		N	14	14	14	14	14	14
	White blood cell Count pre operatively	Correlationscoefficient	,221	1,000	,547**	,458*	,298	,311
		Sig. (2 tailed)	,273	.	,009	,027	,203	,134
		N	14	14	14	14	14	14
	Pediatric Appendicitis Score	Correlationscoefficient	,258	,547**	1,000	,909**	,439	,439*
		Sig. (2 tailed)	,219	,009	.	,000	,073	,042
		N	14	14	14	14	14	14
	Alvarado Score	Correlationscoefficient	,219	,458*	,909**	1,000	,449	,371
		Sig. (2 tailed)	,292	,027	,000	.	,064	,082
		N	14	14	14	14	14	14
	Appendicitis grading	Correlationscoefficient	,033	,298	,439	,449	1,000	,295
		Sig. (2 tailed)	,897	,203	,073	,064	.	,224
		N	14	14	14	14	14	14
	Appendix Diameter in Sonography	Correlationscoefficient	,185	,311	,439*	,371	,295	1,000
		Sig. (2 tailed)	,374	,134	,042	,082	,224	.
		N	14	14	14	14	14	14
	Isoprene Mean Adj. 1.	Correlationscoefficient	-,376	-,297	-,175	-,092	-,331	-,357
		Sig. (2 tailed)	,062	,139	,402	,658	,157	,085
		N	14	14	14	14	14	14
	Acetone Mean Adj. 1	Correlationscoefficient	-,110	-,253	-,105	-,092	-,331	-,058
		Sig. (2 tailed)	,584	,208	,615	,658	,157	,781
		N	14	14	14	14	14	14
	Dimethylsulfid Mean Adj. 1	Correlationscoefficient	-,331	-,099	-,058	,023	-,033	-,012
		Sig. (2 tailed)	,100	,622	,780	,912	,888	,956
		N	14	14	14	14	14	14
	Benzene Mean Adj. 1	Correlationscoefficient	,044	,099	-,012	-,023	-,099	,104
		Sig. (2 tailed)	,826	,622	,955	,912	,671	,617
		N	14	14	14	14	14	14

*. Correlation significant at 0.05 (two tailed)

4 Discussion

4.1 General Aspects

This thesis only includes fourteen out of the forty planned patients because the pilot study had to be put on hold due to the worldwide COVID19 crises. Since it was not ethically justifiable to have more members of a team in the operating room than absolutely necessary in this critical situation, the study was discontinued at this point, since the sampling happened during surgery and was handled by an additional team member. Therefore, preliminary results were used for the statistical analysis, which, however, made it possible to show first effects among the chosen samples.

A variety of prior studies have shown that open appendectomies bare a higher risk of wound infection but are conducted faster, while laparoscopic appendectomies might take longer but children need less pain medication afterwards and can return home post-operatively quicker (29, 30, 31). To assess which method of surgery causes less oxidative stress and can then be favored over the other surgical method, volatile organic compounds, known as VOCs, were sampled and evaluated in the present study. These exhaled compounds are a possibility to measure oxidative stress level during various surgical procedure. The production of oxidative stress during an appendectomy is well known (40) but is usually measured by using serum markers. A study from 2016, for example, has compared the oxidative stress level during open versus laparoscopic appendectomies in adults using blood samples. The results showed that serum makers regarding oxidative stress are higher at the end of open appendectomies compared to the laparoscopic approach (39).

The present study, however, used the opportunity to evaluate oxidative stress level via breath analysis. There are a number of studies trying to identify human body´s physiological VOC concentration levels in order to facilitate the comparison to intraoperatively obtained values (38, 44, 45). Four VOCs, acetone, isoprene, benzene and dimethylsulfide were chosen in this pilot study, since those could be

linked to possible effects in similar studies (38, 40, 47, 49, 54). The results of this pilot trial are preliminary but certain tendencies concerning the different concentrations during the surgical procedures could be identified. The concentrations of dimethylsulfide and acetone showed a significant difference between the two surgical methods at the beginning. This did not apply to benzene, but its concentration changes significantly during the laparoscopic appendectomies.

The goal of the pilot study was to evaluate whether one surgical method should be favored over the other due to oxidative stress and if the surgical setting in the future could be optimized by, for example, responding to various oxidative stress levels pre- and post-operatively. It was not yet possible to identify significant differences in the VOC concentrations throughout the two surgery methods, but calculations can be more precise as soon as more samples are included. The question whether an open or a laparoscopic appendectomy poses a higher risk of oxidative stress, cannot be answered with the preliminary results at this point. The changes of concentration during surgeries point in a significant direction and can be seen as hypothesis generating. It is not meaningful to project the results on a larger population because only a small sample size was included but it is possible to identify the first significant results in regards of changing VOC concentration levels, which can then be further investigated.

4.2 Limitations

4.2.1 Breath analysis

The diagnostic possibility of breath analyses regarding volatile organic compounds is not in routine use in clinical centers. Sampling, preconcentration, normalization and analysis of data have been proven to be difficult in this setting. In the sampling procedure problems like dilution, loss of compounds and contamination with dead space gas makes it difficult to establish a standard set of concentration of various compounds. There are two ways to sample the breath, either take a mixed expiratory sample, which is much simpler to collect, or an alveolar sample, which would show a much higher concentration of compounds since it is not diluted by dead space gas and has a lower rate of contamination. In order for the compounds to be detectable, they have to be preconcentrated, since their concentration is in a nmol/l-pmol/l range. This can be achieved by sorbent traps, coated fibers or direct cryofocussation (40). As mentioned above, there are no standardized VOC values, to which one can compare the study results to. At this point in time the volatile organic compound spectrum of human breath is not yet completely analyzed and listed, which made it difficult to assess the obtained values of this pilot study.

4.2.2 Statistical analysis

Only preliminary results of fourteen patients were included in this thesis. This sample number is rather small and therefore normal distribution is difficult to achieve. It is also not possible to count on the central limit theorem with this sample size. Therefore, a number of non-parametric statistical tests had to be applied, since the criteria like a normal distribution could not be met. Non-parametric statistical tests rank data and through that, the impact in regards of outlier is reduced but at the same time information about the strength of differences between collected sample values is lost. Therefore, non-parametric tests tend to have less power than parametric tests. This means, that it is less likely to identify a genuine effect in a collected data set when using non-parametric tests compared to parametric tests,

when tested on a normal distributed data set. Another limitation is the absence of equivalent parametric and non-parametric statistical tests. A counterpart can be found for a number of non-normally distributed data, such as the Mann-Whitney-U test instead of the t-test or the Spearmann Correlation instead of the Pearson Correlation, but there are a few significant calculations, which lack comparable non-parametric tests, such as the mixed analysis of variance (ANOVA) with repeated measures for which a non-parametric counterpart is not available in SPSS 26.0® (62).

5 Conclusion

Analyzing VOCs via breath sampling is still a relatively new technique and not yet implemented in the daily clinical routine. Not only is there no standard sampling method available yet, but there are also no reliably reference values of VOCs due to the complexity of compounds in a human´s breath sample. There is already a considerable amount of research on oxidative stress during clinical procedures but the connection to VOCs and their predictive ability has not been explored thoroughly yet. This pilot study investigated four VOCs and their concentration changes throughout two methods of appendectomies. The results were interpreted exploratively and were meant to act as hypothesis generating. Despite the small sample size of 14 patients, preliminary results could be seen as promising, due to significant differences among VOC concentrations at certain time points. The study will continue, and more patients can therefore be included in the final statistical analyzes, which will possibly lead to even more powerful significant outcomes among the two surgery methods, making visible which appendectomy approach produces less oxidative stress.

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