Small bowel obstruction and toxicity of a new model of adhesion prevention

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DOCTORAL DISSERTATION

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Title and subtitle Small bowel obstruction and toxicity of a new model of adhesion prevention

Abstract *Background*: Small bowel obstruction (SBO) is a common surgical diagnosis. If no signs of strangulation are evident, the majority of the patients can be conservatively managed. Approximately one third of the patients need surgical treatment and there is a need for early parameters that can predict operative intervention. The etiology of SBO in most cases is postoperative abdominal adhesions. Laparoscopic surgery generates less surgical trauma and thereby possibly less adhesion formation and subsequent decreased risk of SBO. The burden of postoperative adhesions is substantial and there is a need for an adhesion preventive agent that can be used in different clinical situations and reduce the clinical complications caused by adhesions, ie SBO, infertility and chronic abdominal pain. Previous experimental studies have reported promising anti adhesive effect of intraabdominally installed differently charged polypeptides in different clinical settings. However, there was observed toxicity of the cationic polypeptide when administered alone. *Aims/methods*: The aims of the retrospective clinical studies were to identify early parameters predicting surgical intervention in patients with SBO (I) and to determine whether there is a difference in the incidence of SBO after open versus laparoscopic surgery for suspected appendicitis (II). In the experimental studies the aims were to establish the lowest anti adhesive dose of α-polylysine (PL) in combination with polyglutamate (PG) and determine the toxic dose of α-PL (III), to investigate the possible anti adhesive effect of another four cationic polypeptides in combination with PG (IV). Furthermore, explore the mechanism of toxicity as well as the biodistribution of α-PL, alone or in combination with PG, after intravenous and intraperitoneal administration (V).

Results/conclusions: 109 patients were included, 65 were conservatively managed and 44 were surgically treated. We identified five parameters, possible to retrieve within 4 hours from hospital admission, that were more frequent in the patients that were surgically treated for SBO. These parameters can possibly be used to advance the selection of patients for operation (I). The incidence of SBO after open and laparoscopic surgery for suspected appendicitis was low in both groups, 1% (24/2333) and 0,4% (10/2372) respectively. The difference was minor but significant, favoring the laparoscopic approach (II). We could show that the anti adhesive effect of α-PL/PG was dose dependent and the lowest effective dose for α-PL was established. The toxic dose of α-PL was determined and the gap between the lowest effective dose and the toxic dose is probably too narrow (III). All four alternative cationic polypeptides (polyarginine, lactoferrin, lysozyme,ε-PL) investigated in the fourth study showed anti adhesive effect. ε-PL, another isoform of PL, was superior to the other three and showed less toxicity than α-PL (IV). High doses of intravenous α-PL caused a damage to endothelial cells with subsequent edema and extravasation of blood in lung and liver. The biodistribution and accumulation of α-PL and α-PL/PG in blood and organs is lower and slower after intraperitoneal than intravenous administration (V).

Key words: small bowel obstruction, predictive parameters, appendectomy, open versus laparoscopic, abdominal adhesions, prevention, polypeptides, toxicity, biodistribution

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To the "three musketeers", my beloved sons, Hjalmar, Sixten and Malte

Contents

Original papers

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals. The papers are appended at the end of the thesis.

I. Isaksson K, Weber E, Andersson R, Tingstedt B. Small Bowel obstruction: early parameters predicting the need for surgical intervention. *Eur J Trauma Emerg Surg 2011;37:155-159*.

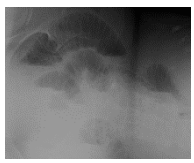
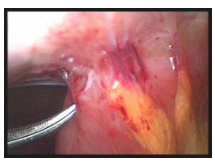
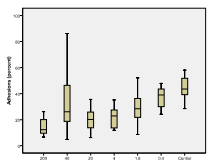
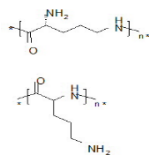
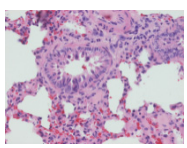
II. Isaksson K, Montgomery A, Moberg A-C, Andersson R, Tingstedt B. Long-term follow-up for adhesive small bowel obstruction after open versus laparoscopic surgery for suspected appendicitis. *Ann Surg, Epub ahead of print, Dec 26, 2013.*

III. Isaksson K, Åkerberg D, Andersson R, Tingstedt B. Toxicity and dose response of intra-abdominally administered poly-L-α-lysine and poly-L-glutamate for postoperative adhesion protection. *Eur Surg Res 2010;44:17-22.*

IV. Isaksson K, Åkerberg D, Said K, Tingstedt B. Cationic polypeptides in a concept of oppositely charged polypeptides as prevention of postsurgical intraabdominal adhesions. *J Biomedical Science and Engineering 2011;4:200-206.*

V. Isaksson K, Åkerberg D, Posaric-Bauden M, Andersson R, Tingstedt B. In vivo toxicity and biodistribution of intraperitoneal and intravenous Poly-L-lysine and Poly-L-lysine/Poly-L-glutamate in rats. *J Mater Sci: Mater Med, Epub ahead of print, Jan 22, 2014.*

Thesis at a glance

Study	Aim	Methods/materials	Results/conclusion
I 	To identify possible early parameters that can predict operative intervention for small bowel obstruction (SBO).	Retrospective chart review. 109 patients with SBO who underwent follow-through examination were included, 44 were surgically treated.	No previous surgery, dehydration, CRP>10, no flatulence and differeniated air-fluid levels are early parameters predicting surgery for SBO.
II 	To determine the incidence of SBO after open and laparoscopic surgery for suspected appendicitis in an adult cohort.	Retrospective chart review. 4705 patients were included. 2333 had open surgery and 2372 had laparoscopic surgery done.	The incidence of SBO was low in both groups, 1% in the open group and 0,4% in the laparoscopic group. Independent risk factors for SBO were open surgery and high age.
III 	To investigate whether the anti adhesive effect of α-polylysine (PL) and polyglutamate (PG) is dose dependent and to evaluate toxicity of α-PL.	Experimental surgical adhesion model. 152 mice were used. α-PL/PG was installed and the adhesion evaluation was made after 1 week. For toxicity part, the α-PL was installed alone.	The anti adhesive effect of α-PL/PG is dose dependent. The lowest effective dose was identified and the toxic dose of α-PL was estimated and is probably to close to the lowest effective dose.
IV 	To evaluate the possible anti adhesive effect of another four cationic polypeptides in combination with PG.	Experimental surgical adhesion model. 125 mice were used. The different cationic polypeptides and PG were installed as in paper III.	ε-PL, polyarginin, lysozyme and lactoferrin all showed anti adhesive effect. ε-PL was the most potent and is less toxic than α-PL and needs more research.
V 	To examine the mechanism of toxicity of α-PL and also to chart the biodistribution of α-PL as single treatment and in combination with PG.	Experimental animal study. The α-PL and PG were administered iv or ip. Repeated blood sampling as well as tissue for histology was performed.	Histology revealed a disruption of the endothelial lining, possibly explaining the toxic mechanism. α-PL accumulates in many organs.

Abbrevations

ASBO	adhesive small bowel obstruction
BMC	Biomedical Center
CRP	C-reactive protein
CT	computed tomography
FITC	flourescein isothiocyanate
HA-CMC	hyaluronic acid carboxymethyl cellulose
ICD 9, 10	International Classification of Diseases (9 and 10)
IL-6	interleukin-6
kDa	kilo Dalton
LD50	lethal dose 50 percent
LG	laparoscopic group
MRI	magnetic resonance imaging
NMRI	Naval Medical Research Institute
NOG	non operated group
OG	operated group in paper I and open group in paper II
PAI	plasminogen activator inhibitor
PG	poly-L-glutamate
PL	poly-L-lysine
ROS	Reactive Oxygen Species
SBO	small bowel obstruction
SCAR	Surgical and Clinical Adhesive Research group
SD	standard deviation
TGFβ	transforming growth factor beta
tPA/uPA	tissue type plasminogen activator/ urokinas plasminogen activator
US	ultrasonography
vWf	von Willebrand factor
α-PL, ε-PL	alpha poly-L-lysine, epsilon poly-L-lysine

Populärvetenskaplig sammanfattning

Tarmvred är en vanlig kirurgisk åkomma som föranleder sjukhusvård. Tarmvred orsakar försvårad eller helt upphörd passage av tarminnehållet på grund av att det är trångt på ett eller flera ställen på tarmen. De vanligaste symptomen på tarmvred är uppspänd buk, illamående och kräkningar, och krampartade buksmärtor. Gasavgång och avföring upphör vanligen. Tarmvred drabbar oftast tunntarmen och orsakerna kan vara flera. Inre ärrbildning, sammanväxningar, som har uppkommit efter tidigare bukoperationer är den absolut vanligaste orsaken till tunntarmsvred och ansvarar för upp till 70% av alla tunntarmsvred som behöver opereras. Det kan vara svårt att handlägga patienter med tunntarmsvred och det viktigaste är att besluta om en operation behövs eller inte. Vanligen utförs en konstrastströntgen med upprepade bildtagningar för att bestämma huruvida en patient behöver opereras eller om patienten kan behandlas konservativt, dvs utan operation. Det tar ofta lång tid från det att patienten har kommit in på sjukhus tills det att beslut om eventuell operation tas.

Sammanväxningar uppstår efter operationer i buken som ett naturligt led i läkningsförloppet efter kirurgi. Sammanväxningarna tillbakabildas hos de allra flesta men hos en del patienter kvarstår de och kan orsaka just tarmvred men även kvinnlig infertilitet samt kronisk buk och bäckensmärta. Sammanväxningar orsakar stort lidande för de patienter som drabbas av dess komplikationer och föranleder dessutom en mycket hög kostnad för sjukvården och samhället. I Sverige är kostnaden beräknad till ca 500 miljoner kronor per år.

Bukkirurgi görs med både öppen teknik eller titthålsteknik (laparoskopisk). Laparoskopiska operationer har de senaste decennierna blivit allt vanligare och är numera den teknik man väljer i första hand vid många kirurgiska diagnoser. När man har jämfört öppen operation och laparoskopisk operation i flera tidigare studier, har man kunnat visa på flera fördelar med den laparoskopiska tekniken. Mindre smärta efter operationen, lägre risk för sårinfektion, kortare vårdtid, kortare tid till återhämtning är några positiva effekter som har noterats. Laparoskopisk teknik orsakar mindre skada än öppen teknik på bukhinnan vilken omger bukhålan och merparten av organen i buken. Detta i sin tur tros minska risken för att utveckla sammanväxningar efter operation.

Blindtarmsinflammation (appendicit) är vanligt med en risk på 7-9 % att insjukna genom livet. Diagnosen är vanligast i ungdomen men kan drabba både små barn och äldre. Appendicit behandlas vanligen med att blindtarmen opereras bort (sk

appendektomi), antingen med öppen operation eller via laparoskopisk operation. Det har visats i studier på barn att det är färre som insjuknar i tarmvred, orsakat av sammanväxningar, efter laparoskopisk appendektomi jämfört med öppen appendektomi. Vid den laparoskopiska tekniken finns också en bra möjlighet att lämna kvar en till synes frisk blindtarm och söka efter annan diagnos.

Vid operation är det viktigt att hantera vävnaderna varsamt, vara noga med blodstillning, undvika uttorkning av bukhinnan och använda material som inte irriterar, allt för att minska risken för att bestående sammanväxningar skall bildas. Även om detta görs kommer problemet med sammanväxningar inte att upphöra. Det finns ett fåtal produkter på marknaden som idag är godkända för att använda i buken i samband med operation för att minska risken för sammanväxningar. Dessa preparat har dock vissa begränsningar och har inte klart kunnat visa att de kliniska komplikationerna av sammanväxningar såsom tarmvred, infertilitet och kronisk buk- och bäckensmärta minskar.

I delarbete **I** ville vi undersöka om det fanns några faktorer hos patienter med tarmvred som kunde återfinnas redan inom några timmar efter ankomst till sjukhus, vilka i sin tur kunde förutse om operation behövdes. Vi fann att ingen tidigare bukoperation, förekomst av ett specifikt röntgenfynd, förhöjd akut inflammatorisk sänka, tecken på uttorkning och frånvaro av gasavgång, var parametrar som var vanligare hos de operativt behandlade patienterna och därmed kan förutse operationsbehovet.

I delarbete **II** var syftet att studera om det var någon skillnad i återinsjuknande i tarmvred efter öppen respektive laparoskopisk operation för misstänkt appendicit. I en stor journalstudie innefattande nästan 5000 patienter, varav ca hälften var öppet opererade och hälften laparoskopiskt opererade, fann vi att risken för tarmvred efter båda operationsteknikerna var låg men att den var ännu lägre i den laparoskopiska gruppen.

Forskargruppen har i flera tidigare experimentella djurstudier visat på att behandling med kombination av två olikladdade sk polypeptider minskar utvecklingen av sammanväxningar efter bukkirurgi på mus och råtta. Polypeptiderna bildar tillsammans ett vävnadsvänligt ”internt plåster” på skadad bukhinna. En av de ingående polypeptiderna (α-polylysin) har visat på negativa (toxiska) effekter när det används ensamt vilket föranledde delarbete **III-V**. Vi har i dessa djurstudier kunnat visa att kombinationen av de olikladdade polypeptiderna följer ett sk dos respons mönster, dvs lägre dos ger sämre effekt på minskningen av sammanväxningar. Vi har också kunnat identifiera den lägsta effektiva dosen och därmed kommit långt ifrån den dos som i försöken uppvisade toxicitet. Vi undersökte även andra polypeptider som uppvisade olika grad av god reduktion av sammanväxningar. Slutligen har vi möjligen klarlagt på vilket sätt α-polylysin utövar sin toxicitet. α-polysinet verkar skada cellerna i kärlväggen så att blödning och vätskeutträde sker.

Foreword

"A male patient, aged 45, is referred to the surgical ward due to suspected small bowel obstruction. He is suffering from colicky pain, distended abdomen, vomiting, constipation and absence of flatulence. Abdominal radiography confirms the diagnosis of small bowel obstruction with dilated small bowel, several air fluid levels and no colonic gas. He has no clinical signs of suspected strangulation and the surgeon on call plan for a conservative regime. Parallel to intravenous fluid, a nasogastric tube is prescribed, and a follow through examination with water-soluble contrast medium is started.

In the patient chart it is revealed that 10 years ago he had an open appendectomy done, both the macroscopically and histopathological examination of the appendix turned out negative. He was discharged with the diagnosis of non specific abdominal pain. About one year later he had his first episode of small bowel obstruction and was surgically treated by laparotomy for an adhesional band. Last year he suffered his second episode of adhesive small bowel obstruction that needed surgical treatment after initially failed conservative treatment. The operation was time consuming, the extent of adhesions was significant and three inadvertent enterotomies resulted in a short small bowel resection. Postoperativelly he suffered from prolonged ileus and stayed in hospital for 10 days. He could return to his job as a bus driver after 4 weeks."

The fictional case report above is the everyday reality for many patients and surgeons worldwide. Small bowel obstruction is one of the most common surgical diagnoses and the cause is postoperative abdominal adhesions in the majority of the patients. Adhesions are a consequence of most abdominal surgery but most frequently following surgery to the lower abdomen, including colorectal and gynecological surgery as well as appendectomy. Postoperative adhesions cause, besides small bowel obstruction, chronic pelvic and abdominal pain, secondary female infertility and result in higher risk for complications in subsequent surgery. Furthermore, adhesions cause a significant health care problem with high morbidity and do also account for a major financial burden. The best treatment for adhesions is their prevention. The need for an optimal adhesion prevention agent that could be used in every clinical setting and with significant reduction of the clinical manifestations due to postoperative adhesions is urgent.

Introduction

Small bowel obstruction

Background

Acute bowel obstruction is a leading cause of unscheduled surgical admissions and is one of the most frequent causes of emergency operations. Small bowel obstruction (SBO) comprises the majority of all bowel obstruction. Approximately 75 % of the patients diagnosed with mechanical bowel obstruction in an observational study by Markogiannakis [1], had obstruction of the small bowel. The cardinal symptoms and clinical findings of SBO are abdominal distention, colicky pain, nausea/vomiting and absence of passage of flatus and feces. The symptoms can be differently presented in different individuals and not all of the patients have all the signs described above or in addition have other more alarming signs of SBO such as persistent pain with or without peritonitis, suggesting ischemic complications. SBO is a surgical diagnosis that does not respect gender or age and is accompanied with high morbidity and financial expenditures globally.

Etiology

Adhesions, volvulus of the small intestine, hernia, Crohn´s disease, gallstones, Meckel´s diverticulum, intussusception, radiation injury and neoplasms are many but not all underlying etiologies for SBO. The most common cause of SBO is abdominal adhesions which in turn predominantly are secondary to previous abdominal surgery. Postsurgical adhesions, multiple matted or single bands, are responsible for up to 75 % of those SBO patients treated operatively[2-4]. In particular, surgery to the lower abdomen, e.g. colorectal surgery including appendectomy and gynecological surgery, is prone to result in permanent adhesions with subsequent high risk for SBO. The risk of SBO following surgery to the lower abdomen is approximately doubled compared to upper abdominal surgery[5]. The reason for this is thought to be the position of the small intestines in the lower abdomen. Colorectal operations, including appendectomies, are stated in the literature to account for up to 50 % of the SBO cases and the risk for postoperative adhesive SBO (ASBO) is reported very high following specific procedures[6]. Ileo-pouch anal anastomosis surgery is afflicted with the highest numbers

of SBO. Fazio et al reported in a study from 1995 a risk of 25 % and in a recently published study they state that the SBO rate is 18% after this procedure[7,8]. In a study from the Mayo Clinic, episodes of SBO occurred in approximately 35-40 % at 20 years after ileo-pouch anal anastomosis[9].

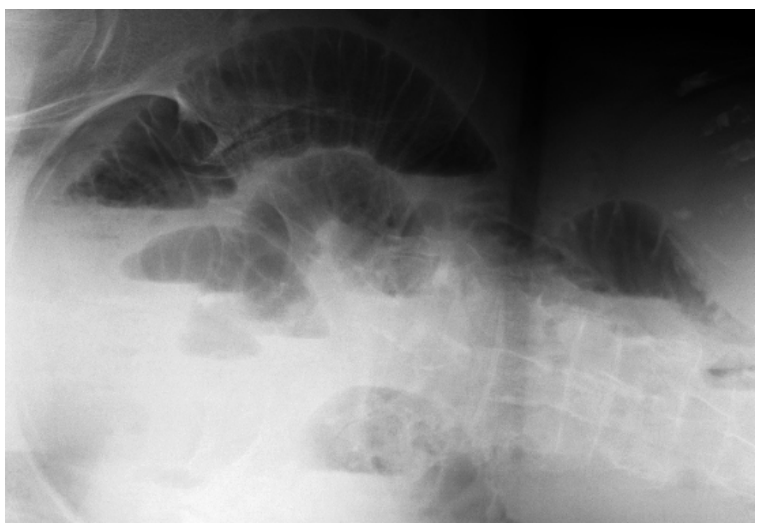
Management

Patients with SBO require careful and appropriate management and are often difficult to assess even for an experienced surgeon. A proper diagnostic and therapeutic strategy is important. The decision of whether the obstruction is complete or partial is crucial, the former needing surgical intervention and the latter is often successfully managed conservatively. Approximately one third of the patients with SBO will need operative treatment[1,10]. The choice between operative or non operative treatment of SBO is difficult and some surgeons proclaim early surgery for most patients while others advocate a conservative strategy in the majority of patients. If there is suspicion of strangulation, immediate surgery should be done without any delay.

Diagnostic pathway

Besides a detailed patient history, including information of possible previous abdominal surgery, a meticulous clinical examination and evaluation of laboratory parameters, the suspicion of SBO should be confirmed with radiology. Conventional plain abdominal radiography (Fig. 1), computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography (US) are possible radiological methods that all have different potentials and accessibility concerning SBO.

Figure 1. A plain abdominal film of small bowel obstruction.



Both MRI and US are diagnostic tools without ionized radiation but have not gained success in the everyday routine in the suspicion of SBO. Though US is a highly user dependent method, it has the ability to detect free fluid between dilated small bowel loops. If there is evidence of a large amount of fluid this indicates a more severe obstruction which predicts surgical treatment [11].

CT is an imaging modality which is highly diagnostic in SBO and has the ability to detect the possible cause of a complete obstruction. Furthermore, CT has the potential of assessing the presence or absence of intestinal ischemia with very high sensitivity and specificity[12,13].

Despite the lower sensitivity and specificity, plain abdominal film remains the most useful non invasive procedure in radiologic diagnosis of SBO. Recently updated guidelines for diagnosis and management of ASBO, recommend plain abdominal radiography to be the initial radiological evaluation and CT scan should be preserved for secondary evaluation when the plain film is not conclusive for SBO[14,15].

A low dose CT is an alternative to plain abdominal film and has become the leading initial radiological evaluation for patients with acute abdomen in many hospitals. The low dose CT is performed without intravenous or oral contrast and the radiation dose is reduced compared to regular CT and almost equal to the dose for plain abdominal film.

A follow through examination with water-soluble contrast medium is a well established method to decide whether the obstruction is complete or partial. This can be performed if the patient is not considered for immediate operation due to suspicion of strangulation or obvious causes of the obstruction other than adhesions[16-18].

Therapeutic pathways

Patients suffering from SBO will benefit from a nasogastric tube for drainage and decompression parallel to intravenous fluid resuscitation and correction of imbalances of electrolytes, if present. Careful and frequent clinical reassessment is crucial to be aware of any changes in status.

Non operative treatment

If there is no suspicion of strangulation, most patients with SBO can safely start an initial conservative trial[19]. Fevang et al concluded that a non operative treatment resulted in a high resolution rate (64 %) of the obstruction and the overall morbidity and mortality was low for patients with no signs of strangulation[19]. Follow through examination with water-soluble contrast medium is primary a diagnostic tool to decide partial or complete obstruction though the administration of water-soluble contrast is shown to have therapeutic properties. Gastrografin®, the most widely used water-soluble contrast medium in SBO, is a hyperosmolar mixture of sodium diatrizoate and meglumin diatrizoat. The osmolarity is almost six times the osmolarity of extracellular fluid and this promotes fluid into the intestinal lumen diluting the bowel contents and

thereby increasing the pressure. The fluid transition also reduces the edema of the intestinal wall, resulting in better contractility capacity and together with the higher intraluminal pressure, the passage through the obstructed site is facilitated. If the contrast medium does not reach the colon within 24 hours the SBO is less likely to resolve. The use of Gastrografin® reduce resolution time and hospital stay[16,20-22] and some authors state its favor in reducing the need of surgery[16,20] and other studies do not report a reduced risk for surgery[22]. Hyperbaric oxygen therapy is another non operative treatment of ASBO that has been reported in the literature[23] but the availability of the treatment must be questioned. The Bologna Guidelines argue that this could be an option in the management for whom surgery should be avoided[14]. Patients treated conservatively have shorter time to recurrence of SBO episodes[10,24,25].

Operative treatment

If conservative treatment is not successful, i.e. no progress in the follow through examination or worsening of the clinical appearance of the patient, surgical intervention is inevitable. Chen et al demonstrated in a study that 96 % of patients with ASBO in whom the Gastrografin® had not reached the colon within 24 hours required surgical treatment[26].

As mentioned above, the most common cause of SBO is postoperative adhesions. Abdominal adhesions are accompanied with high morbidity at subsequent operations, resulting in longer operating time, increased risk for inadvertent enterotomies and bowel resection[27-30]. Open or laparoscopic approach is of course a question of expertise, though laparoscopic technique for SBO and especially adhesive SBO should be used carefully. The risk for missed and delayed diagnosis of peroperative inadvertent enterotomies is higher in laparoscopic adhesiolysis compared to open and, moreover, the conversion rate to open laparotomy is frequent[14,31].

Williams et al showed that operatively treated patients had lower recurrence frequency and a longer time interval to recurrence of SBO compared to conservatively treated patients. They had, however, longer hospital stay[24]. Matted adhesions, age below 40 and postoperative surgical complications increase the risk for recurrence of SBO following surgery for ASBO[25,32]. The description of matted adhesions is found in the following section separately discussing adhesions.

Predictive factors

One of the disadvantages of the follow-through examination is the time required to determine whether the obstruction is complete or not. It is generally agreed that a delay in treatment of SBO increase the risk of prolonged hostpital stay, complications and death[33-35]. There is a need for more predictive factors that are available early on and can discriminate patients in need for surgery. Leung et al identified younger age, no previous abdominal operation and absence of adhesive disease as factors predicting

surgical treatment[35]. In a recent study, Cosse et al demonstrated that elevated levels of serum procalcitonin may be useful to predict failure of conservative management of SBO and also the occurrence of bowel ischemia[36]. Progressive increase in bowel wall thickening, revealed with ultrasonographic examination, is associated with higher risk for surgical management[37]. Lappas et al showed that the presence of air-fluid levels of differential height in the same dilated small bowel loop and the presence of a mean air-fluid width greater or equal to 25 mm on abdominal radiograph indicate high grade or complete obstruction[38]. These findings were confirmed by Thompson et al in a study where the accuracy of plain abdominal radiography for SBO was examined[39].

Appendicitis

Background

Appendicitis is a common differential diagnosis in patients presenting with lower abdominal pain. The life time risk of acute appendicitis is 7-9 %[40]. The incidence peaks in adolescence and the diagnosis is slightly more common in men[40-42]. Acute appendicitis has been reported to have a seasonal trend with higher incidence during summer months[42]. The natural course of appendicitis is not clearly evaluated though spontaneous resolution of appendicitis is thought to be common[43]. The prognosis of appendicitis is in particular dependent on the grade of inflammation. The morbidity and mortality is substantially increased with perforated appendicitis[44-46]. The incidence of perforated appendicitis is reported to be 8-25 %[47-51]. The rate of perforation is not significantly increased by delayed surgery and the majority of perforations are thought to be present already at patient admission[41,43,52]. Other authors proclaim the reverse. Busch et al demonstrated that an in-hospital delay of more than 12 hours was an independent risk factor for perforation[50].

Management

Clinical assessment including basic laboratory tests evaluating inflammatory response is important. The use of clinical scoring systems has been proposed to raise the diagnostic accuracy for appendicitis. Alvarado score[53] and appendicitis inflammatory response (AIR) score[54] are both scoring systems that are shown to have high accuracy. Clinical scoring systems have the ability to discriminate patients to groups of different probability levels of appendicitis and thereby identifying patients for immediate surgery, observation at home or further investigations [53-56]. Scoring systems may seem attractive, but they have not gained wide success.

Imaging techniques, US and CT, are frequently used nowadays for suspected appendicitis and are shown to reduce the rate of negative surgical explorations if used

with correct indications[57-59]. Both investigations have disadvantages, US is highly examiner dependent and CT is accompanied with potentially harmful ionized radiation. The radiation dose should be especially considered since the majority of the patients suffering from suspected appendicitis are young. The risk of high false-positive and false-negative diagnosis should be considered when imaging is used as a screening tool, thus CT and US for suspected appendicitis should be used selectively[56,60].

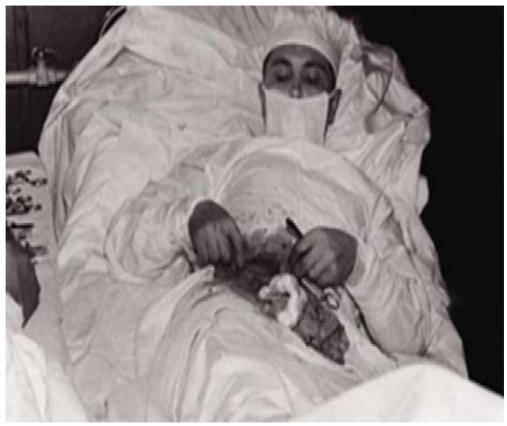
Treatment

The interest in conservative antibiotic treatment for acute appendicitis has increased during the last decade. Several studies have shown evidence of successful antibiotic treatment but with accompanying unknown long term recurrence risk[61-63]. Ansaloni et al concluded in a systematic review and meta-analysis of randomized controlled trials concerning antibiotic treatment for appendicitis, that although a non surgical approach can reduce the complication rate compared to appendectomy, the lower efficacy prevents antibiotic treatment from being a viable alternative to surgery[64].

Appendectomy

The standard treatment of appendicitis, for most surgeons, is appendectomy. Appendectomy is one of the most common emergency operations worldwide. The life time risk for appendectomy is 12 % for males and 23 % for females[40]. Approximately 10 000 appendectomies are performed annually in Sweden, the corresponding number in the United States is approximately 250 000[40,65]. Appendectomy is considered to be a safe surgical procedure with low mortality rate [46,66,67]. Some surgeons advocate aggressive surgical approach while others favor a high threshold for operation. The argument for aggressive surgical manner is to minimize the risk of perforation whereas those who proclaim the other attitude intend to avoid negative appendectomy. The rate of negative appendectomy is reported in the literature from 10 to 23 %[44,48,51,68,69] and a gradual decrease has been observed with time[42,48,70,71]. However, Flum et al could not show a reduction of the rate of negative appendectomy despite increased availability of diagnostic tools[51].

Figure 2. Open appendectomy – "auto-appendectomy"



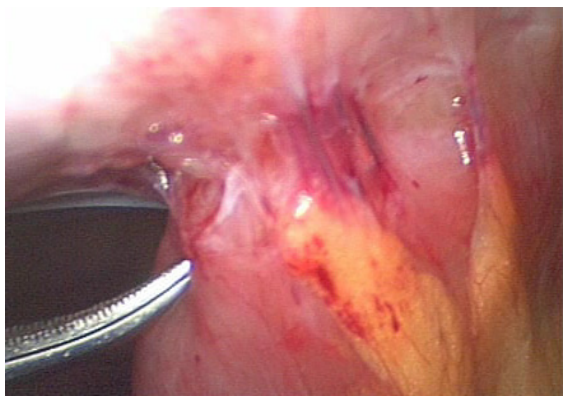
Leonid Rogozov diagnosed himself with peritonitis and suspected appendicitis during the Soviet Antarctic Expedition in 1960-61. He performed an open appendectomy, the procedure was carried out in local anesthesia. A driver, a meteorologist and a mirror were his assistants[72].

Conventional open appendectomy by a muscle splitting incision in the right fossa, first described by Mc Burney in 1894[73] (Fig. 2), has been the gold standard until Semm, a German gynecologist, performed the first laparoscopic appendectomy in 1983[74]. Since then, this approach has gained popularity in pediatric as well as adult patients. Several studies have described advantages using the laparoscopic technique including diagnostic possibilities, faster recovery with shortened hospital stay, decreased postoperative pain and lower incidence of wound infections[75-77]. A Cochrane analysis by Sauerland et al report higher risk of intraabdominal abscess formation following laparoscopic appendectomy[75] while the meta analysis by Wei et al could not demonstrate any difference between open and laparoscopic appendectomy[78]. Diagnostic laparoscopy is a safe procedure with high accuracy and low rate of complications (0-1,4 %)[79-81] and offers the surgeon a superior possibility to explore the abdomen for other diagnoses than appendicitis. The procedure gives the opportunity to safely leave a macroscopically normal appendix in place[79,82,83].

Faiz et al showed, in a study from England, a lower mortality following laparoscopic appendectomy compared with open appendectomy, a one year mortality rate of 0,29 % and 0,64 % respectively[67]. This finding is endorsed by other authors[76,84,85]. However, the results from those studies have been argued due to that conversions from laparoscopic to open appendectomy have been allocated to the open surgery group not analyzed according to intention to treat and not adjusted for co-morbidity[86]. Perforated appendicitis and negative appendectomy is accompanied with higher mortality rate[46,67,68,86].

The incidence of SBO following appendectomy (Fig. 3) is historically reported to be between 0,2 % and 10,7 %[87-91]. In a large Swedish population based study, it was demonstrated that the cumulative risk of surgically treated SBO after open appendectomy was 1,3 % compared to 0,2 % for non operated controls after 30 years of follow-up[45]. The highest risk for SBO was found after operation for perforated appendicitis and negative appendectomy, supported by other authors[44,45,47]. The risk of SBO after appendectomy is in most recent reports low. However, appendicitis is one of the most common surgical diagnoses and since appendectomy constitutes the most common emergency general surgical operation, the actual number of patients who will suffer from SBO will be substantial.

Figure 3. Laparoscopy revealing adhesions after open appendectomy, the cause of SBO in this case.



The extent of surgical trauma is thought to have impact on the development of adhesion formation and the use of laparoscopic technique cause less adhesions. Several studies have been carried out concerning the incidence of SBO following open versus laparoscopic appendectomy. The risk of SBO is shown to be less with the less traumatic laparoscopic approach in pediatric surgery[92,93]. In a systematic review, Markar et al showed a decreased risk for SBO following laparoscopic appendectomy for complicated but not for uncomplicated appendicitis in children[94]. The advantage of laparoscopic appendectomy in decreasing the risk for SBO in adult patients has not been clearly demonstrated. Some authors proclaim a reduced risk[84,95] while others have demonstrated no difference between the two surgical approaches[47,96-98]. In fact, in the study by Swank et al, with a limited follow up of 3 years, they did not register any patient with postoperative SBO[96]. The diversity of methodology and length of follow up in studies comparing postoperative complications, including SBO, following laparoscopic versus open appendectomy makes it difficult to generalize in either direction.

Adhesions

Peritoneum

The peritoneum is one of the largest human organs with a surface of approximately 10 000 cm^2, an area almost equal to the skin[99]. It is a serous membrane that forms the lining of the abdominal cavity and covers most of the intraabdominal organs. The peritoneum consists of a single layer of mesothelium overlying a basement membrane which in turn is supported by a loose layer of connective tissue underneath[100]. The mesothelial cells are covered with microvilli which increase the peritoneal surface. The connective tissue contains capillary and lymphatic networks. The main functions of the peritoneum are secretion and absorption of fluid and of proteins.

A thin surfactant film, rich in phospholipids, is covering the mesothelial surface and serves to minimize the friction between the abdominal viscera, thereby enabling their free movement[101-104]. The peritoneal fluid contains many of the proteins found in plasma but in a lower concentration. The fluid also contains a variety of cells, e.g. lymphocytes, macrophages, polymorphnuclear cells and free mesothelial cells, which have a crucial role in the peritoneal response to inflammation and surgical trauma. The total volume of peritoneal fluid in the abdominal cavity is normally approximately 5 ml, a smaller elevation is seen in women in the middle of the menstruation cycle[105].

Adhesion formation

Surgical trauma, intraabdominal and pelvic infectious and inflammatory conditions as well as endometriosis can result in peritoneal injury and subsequent development of adhesions. The peritoneum has a remarkable and unique property in restoration. Irrespective of the size of the injury, the peritoneal re-mesothelialization is completed within a week. The peritoneal defect is repaired simultaneously from the entire surface and differs from the gradually healing from the borders in a centripetal manner seen in skin wounds[106,107].

The surgical damage to the peritoneum by mechanical trauma, desiccation, cooling or heating, causes a denudation of the mesothelial layer and exposure of the basal membrane. This initiates a local inflammatory response and an activation of the coagulation cascade[108]. The transformation of prothrombin to thrombin in turn activates the conversion of fibrinogen to fibrin. The formation of fibrin is a natural response in tissue repair and should play a temporary role until normal tissue structure and function is restored. Thereafter the fibrin is supposed to be degraded by plasmin through the fibrinolytic system. Plasmin is derived from plasminogen by plasminogen activators, mainly tPA and uPA (tissue type/urokinas plasminogen activator, respectively). Plasminogen activator inhibitor 1 (PAI 1) is the main inhibitor of the

fibrinolytic pathway. An imbalance in the fibrinolysis is thought to be a main causal factor behind the development of organized permanent adhesions.

The fibrinolytic response is affected and reduced by conventional surgery[109]. The perioperative tPA activity decreases in most types of surgery[110-113] except for short-term laparoscopic surgery that did not induce any fibrinolytic changes[114,115]. Either decreasing levels of tPA or increased levels of PAI 1 results in a reduction of fibrinolytic activity.

Ischemia as a result of peritoneal damage is known to be determinant factors in the formation of adhesions[116]. The role of blood in the peritoneal cavity in the formation of adhesions is controversial[117]. Ryan et al[118] implied that blood plays an important part in the pathogenesis. Clotted blood may constitute a fibrinous network attracting fibroblasts to proliferate with subsequent adhesion formation. Blood in conjunction with peritoneal damage is proposed to be more important than the blood per se in the risk of adhesion formation[118,119].

Permanent adhesions occur as diffuse matted adhesions or as single bands. They can also be classified as de novo or reformatted when they are a result from previous surgery. De novo are defined as new adhesions in previously adhesion free sites and reformed are defined as recurrence when located at the same surgical site after adhesiolysis. Single band adhesions are more common operative findings in patients with no previous history of abdominal surgery[120].

Lower abdominal surgery is a risk factor for adhesion formation. No preoperative laboratory parameters to predict adhesion formation are clinically available. Ivarsson et al showed that patients with high propensity for adhesion development had significantly higher levels of peritoneal PAI-1 compared to patients with less severe adhesions but could not detect any corresponding correlation in peripheral blood[112]. The findings from that study suggest that components of the fibrinolytic system could possibly be used as intraoperativelly tissue markers to identify high risk patients who would benefit from adjuvant anti adhesive treatment.

Clinical and financial aspects

Adhesions are most commonly a consequence of abdominal and pelvic surgery even though infectious conditions and endometriosis are other underlying causes to adhesion formation. Congenital adhesions are a more rare etiology. Adhesions are afflicted with a high impact on healthcare worldwide. Adhesions are reported to be found in 67-93 % of patients who have had abdominal surgery[121,122]. Most adhesions are not symptomatic but in a minority of the affected patients they can result in considerable morbidity. The problem of abdominal complications related to postoperative adhesions is substantial. Ellis et al showed in a large follow-up study, that one third of the patients that had undergone open abdominal or gynecological surgery, were readmitted in mean

2,1 times directly or possibly related to adhesions. 5,7 % of the readmissions were classified as directly related to adhesions and 3,8 % were surgically managed[5].

As depicted previously, adhesions are the major cause of *SBO*, accounting for approximately 70 % of all SBO cases. Almost 1% of all surgical admissions and 3,3 % of all laparotomies in England, over a 25 year period, were due to ASBO[122]. The time relapsing from index surgery to eventual episode of obstruction varies widely. One to ten percent present with an early SBO within the first month, 20-50 % occur within the first postoperative year and up to as many as 10 % will suffer from their first SBO episode as long as 20 years after the initial surgery[25,122-124]. The number of previous episodes of SBO that the patient has experienced is the strongest predictor for recurrence[123]. ASBO and the hazards associated with the need of subsequent surgery are more extensively discussed in the previous section of small bowel obstruction.

Postoperative adhesions are also an important gynecological issue. Adhesions are reported to be the cause of *female infertility* in 15 % up to 30 %[125-127]. The mechanism of reduced fertility caused by adhesions is multi-factorial. The distortion of the fallopian tube by adhesions may result in impaired capability of ovum capture and migration[128]. Nagata et al proposed the possibility of decreased oocyte development and maturation due to ischemia caused by adhesions entangling or obstructing the blood supply[129]. Peritubal adhesions may affect the motility of the tuba and delay or prevent the transport of the embryo to the uterine cavity and this can result in an ectopic pregnancy[130]. Surgical adhesiolysis can increase the pregnancy rate among previously infertile women[131].

Pelvic and abdominal pain as sequelae from adhesions is commonly reported but is a controversial topic. The pain is thought to arise when there is increased tension, stretching and traction of abdominal and pelvic organs due to the motility restriction caused by the adhesions. This gives rise to stimulation of peritoneal pain receptors. It has been suggested that adhesions may contain sensory nerve fibers[132], a possible explanation model of the pain associated with adhesions. There are reports of a reduction in pain in 60-90 % of the cases following adhesiolysis[133]. Paajanen demonstrated pain relief after adhesiolysis and would propose the treatment in selected cases[134].

Other authors are more distrustful to the statement of adhesions as a cause of chronic abdominal and pelvic pain and the relation between adhesions and pain is widely debated[135]. An expression from the eighties, “a myth of that adhesions can cause pain”, belongs to Alexander-Williams[136]. Swank et al designed a prospective trial where patients suffering from chronic abdominal pain and the presence of adhesions were randomized to laparoscopic adhesiolysis or no treatment after an initial diagnostic laparoscopy. The patients as well as the assessors were unaware of the surgical intervention. Both patients that had adhesiolysis performed and those that only had a diagnostic laparoscopy done showed a sustained reduction in pain and a significantly improved quality in life but there was no difference between the two groups. The

conclusion from this study is therefore that laparoscopic adhesiolysis cannot be recommended as a treatment for adhesions in patients with chronic abdominal pain[137]. Since abdominal pain in the general population is a very common symptom[138] and not all patients with adhesions suffer from pain, the question whether adhesions can cause pain or not is a challenge to study.

The *financial burden* of postoperative adhesions is substantial. Several studies have been carried out trying to give a fair picture of the economic impact of adhesion related morbidity. Ray et al reported that adhesiolysis was responsible for more than 300 000 hospitalizations in 1994 with an expenditure of 1,3 billion USD[139]. In Sweden the cost for adhesive bowel obstruction was estimated to 13 million USD[140]. Tingstedt et al demonstrated in another Swedish study from 2007, an approximated annual cost of adhesion-related problems of 40-60 million EUR[120]. The inpatient cost was calculated to be almost equal to the cost of gastric cancer[120]. Kössi et al reported from Finland that the inpatient expenditure of adhesions was comparable to rectal cancer[141]. In a study from Sikirica et al, the inpatient cost for adhesiolysis related procedures in 2005 in the US was calculated to 2,3 billion USD[142]. These studies are differently designed and thereby difficult to compare but the general conclusion is not questioned, namely that adhesions have great impact on health economy worldwide.

Adhesion prevention

To prevent something, you have to be aware of "the something". This remark may seem absurd but two recent studies from The Netherlands, demonstrate that the adhesion awareness of surgeons and gynecologists is limited. Even though postoperative adhesions are the most common postoperative complication, 40 % of the surgeons declared that they never inform the patients about adhesions and only 9,8 % informed the patients routinely. A minority of the gynecologists, 5,2 %, included adhesions in their informed consent on a routine basis[143,144].

Surgical technique

The general intraoperative strategy to limit the formation of adhesions is minimizing the surgical trauma. Gentle tissue handling, prevent desiccation, meticulous hemostasis, avoid spilling of intestinal contents and no use of starch powdered gloves are keystones that should be practiced in all abdominal operations. Unnecessary tissue handling and desiccation increase the risk for more extensive peritoneal injury with mesothelial cell abrasion and ischemia as results, which are potent stimuli for development of adhesions. Dessication of the peritoneum cause a decrease in the peritoneal fluid and lubricating surfactant film covering the peritoneum. This leads to

a desquamation of mesothelial cells and exposure of the underlying basement membrane and gradually fibrin deposition[107].

Experimental studies have demonstrated an increased risk for extensive adhesions when starch powdered gloves are used[145-148]. Extended operation time is another factor influencing the adhesion formation, and furthermore, suturing of the peritoneum should be avoided since this is thought to enhance the formation of adhesions[149,150].

Minimally invasive surgical technique as an alternative to open surgery is applicable for many diagnoses and is reported to reduce the risk of adhesion formation[151]. Angenete et al showed in a large register based study from Sweden, including over 100 000 patients, that open surgery increased the risk for SBO at least 4 times compared with laparoscopic approach for several abdominal and gynecological operations[95]. There are only a few trials evaluating the adhesion formation with a second look operation in humans. Women who had undergone laparotomy because of ectopic pregnancy developed significantly more adhesions at the operated sites than those who had laparoscopic surgery, confirmed by a second look operation[152].

There are several potential advantages with laparoscopic surgery concerning adhesion formation. The total length of incision to the parietal peritoneum is reduced and the presence of adhesion promoting foreign material such as gauze particles and lint from drapes are diminished[153,154]. Retractors in open surgery exert a high pressure on the parietal peritoneum, possible to induce ischemia and subsequent risk for adhesion formation. The use of often more gracile instruments in laparoscopy makes the dissection more precise and possibly gentler tissue handling with reduced manipulation of distant structures from the operative site. There is evidence of faster recovery after laparoscopy with earlier return of bowel motility which. This in turn could be a potential contributory factor to less extent of adhesions due to movements of the bowels inducing mechanical separation of conjunctive peritoneal surfaces.

There are also disadvantages afflicted with laparoscopic surgery. As mentioned above, the laparoscopic approach as well as open surgery induces reduction in fibrinolytic activity with the exception of laparoscopic interventions not exceeding 38 minutes[114], an operating time not applicable for many laparoscopic operations. There are indications that the insufflation of carbon dioxide during laparoscopic surgery can negatively influence the mesothelial lining of the peritoneum; either by increased intraabdominal pressure with peritoneal distention, by the gas flow, the carbon dioxide per se or a combination of all three[155,156]. Gray et al showed that high speed insufflation creates a cooling effect that leads to cell injury[157]. Even though most surgeons are aware of this and use a lower gas flow, we do not know the potential persisting adverse effects from the gas insufflations yet.

Adhesion preventive agents and current status

Despite the efforts made in good surgical manner in the wound and minimally invasive surgery we can just decrease the risk but not prevent postoperative adhesions. Over the last five decades the search for an effective anti adhesive agent has been intense. Several different strategies to approach the problem have been attemped. Anti inflammatory drugs, anti coagulants, ROS scavengers, proteolytic agents, angiogenesis inhibitor, anti estrogen, fibrinolytics, antibiotics and different adhesion preventing barriers have all shown various experimental and/or clinical success, although most of them have not gained clinical acceptance due to limited or even detrimental effects[158,159].

Barriers intend to separate injured peritoneal surfaces from each other, mainly by two different ways, i.e. applying membranes directly onto injured areas or by intraabdominal installation of liquid, a mechanism called hydroflotation. Any preventing strategy should be safe, effective, practical and cost effective. A treatment cost exceeding 200 GBP are unlikely to secure the cost effectiveness on national health care[160].

Four adhesion barriers are in current clinical use. Three of those are approved by the US Food and Drug Administration (FDA); hyaluronic acid carboxymethyl cellulose (Seprafilm®), oxidized regenerated cellulose (Interceed®) and icodextrin 4 % (Adept®). SprayShield® is a polyethylene glycol polymer approved and used in Europe. The European Bologna Guidelines for management of ASBO state that hyaluronic acid/carboxymethyl cellulose and icodextrin 4 % can be used as adhesion preventive agents following surgery for ASBO[14], since they might decrease adhesions, although not decrease the need for surgery. In a recent systematic review and meta-analysis, Interceed was shown to reduce the incidence of adhesions and there were some evidence suggested that Seprafilm® reduced the incidence of reoperations for ASBO[161].

Hyaluronic acid/carboxymethyl cellulose

Hyaluronic acid/carboxymethyl cellulose (HA-CMC), Seprafilm®, is the most widely used and tested adhesion prevention agent. It is a sterile, bioresorbable, translucent adhesion barrier composed of two anionic polysaccharides, sodium hyaluronate and carboxymethyl cellulose. It is indicated for use in patients undergoing abdominal and pelvic laparotomy and is absorbed within 7 days. There are evidence from randomized trials that Seprafilm® reduces the frequency of and severity of adhesions[162]. Seprafilm® has been reported to reduce SBO requiring surgical intervention (1,8 % vs 3,4 %) in a prospective randomized study of 1701 patients with intestinal resection, though no difference was seen in the overall incidence of SBO[163]. In the Cochrane review by Kumar et al, they could not find evidence that Seprafilm® reduce the incidence of SBO or the need for surgical treatment[162]. There are serious abdominal adverse events, including anastomosis dehiscence and abscess formation, reported when Seprafilm® is wrapped around a fresh bowel anastomosis and this is therefore abandoned[164]. Seprafilm® is not possible to use in laparoscopic surgery, it is brittle and some

experience is needed to handle it correctly in open surgery, especially in areas hard to access[165]. Several sheets are often needed and in average approximately 4 sheets are used per operation. The cost will thereby rise per treatment and most probably exceed the cost effectiveness of 200 GBP that Wilson calculated[160].

Oxidized regenerated cellulose

Interceed® is a fabric composed of oxidized regenerated cellulose and is used as adhesion preventive agent in gynecological surgery. It forms a gelatinous protective coat which is absorbed within 2 weeks. It has been shown to be effective in reducing the incidence and extent of adhesions in prospective randomized studies[166,167]. A meta-analysis confirms the results[168]. There is no convincing evidence that Interceed® reduces the incidence of adhesion related clinical outcomes, though there have been possible benefits reported in infertility surgery with an increased conceiving rate after treatment with Interceed® [169]. In experimental animal studies, Interceed® has shown a reduced efficacy in the presence of blood[170] and therefore the hemostasis must be meticulous before application. The use of Interceed® in infectious situations is contraindicated.

Icodextrin 4 %

Icodextrin 4 %, Adept®, consists of glucose polymers and exert adhesion preventive effect by hydroflotation and the fluid is maintained for up to 3-4 days before fully absorbed. Icodextrin 7,5 % has safely been used in patients treated with continuous ambulatory peritoneal dialysis for many years. The trade name of icodextrin 4% is Adept® and experimental studies have shown promising results in adhesion reduction[171-173]. Randomized clinical trials have demonstrated reduced adhesion formation evaluated with second look operation[174,175] and Brown et al also reported improved fertility scores[174]. In a multicenter study, Menzies et al conclude that Adept® has a good safety profile and reported a high satisfaction with ease of use, both in laparotomies and laparoscopies[176]. Catena et al showed, in a recent smaller study, a significant reduced risk of recurrence of ASBO in the group that received Adept® following surgery for ASBO compared to those who were randomized to no treatment[177]. According to US FDA there are contraindications for the use of Adept®; known or suspected allergy to corn starch, the presence of obvious abdomino-pelvic infection, open surgery and procedures involving bowel resection, due to reports of wound complications and anastomotic failure[176]. In the US the use of Adept® is restricted to gynecologic laparoscopic adhesiolysis.

Polyethylene glycol polymer

SprayShield®, a polyethylene glycol polymer, is an anti adhesive agent with relatively scarce evidence of its efficacy due to few clinical studies performed[178,179]. It is preferably used in gynecological laparoscopic surgery. It is applied as a spray which forms a gel when it gets in contact with the peritoneum and functions as a barrier. The solution is

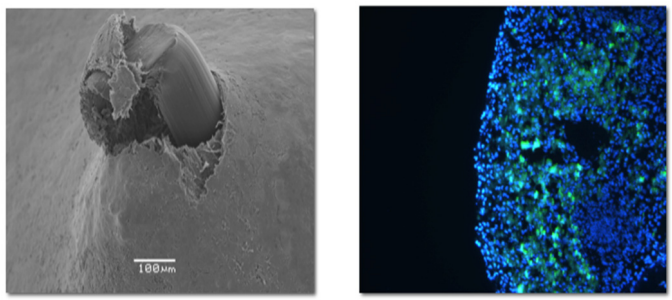
colored offering a visualization of the administered area. The set up is reported complex and time consuming.

Bioactive polypeptides

Polypeptides are being used widely in biological research and are encountered in different settings such as coating of implants, gene vectors, chemotherapeutic drug carriers and antimicrobial and anti neoplastic research[180-185]. Polypeptides in the concept of adhesion prevention are interesting. Bioactive polypeptides are biocompatible and integrate with the peritoneal surface. Previous works by our research group have shown that the combination of two differently charged bioactive polypeptides, poly-L-lysine (PL) and poly-L-glutamate (PG), significantly decrease abdominal adhesion formation in various experimental animal studies[186,187].

The hypothetical mechanism of differently charged polypeptides is based on electrostatic interaction. When the peritoneal surface is injured, negatively charged molecules on mesothelial cells as well as on the submesothelial layer are exposed[188]. PL is a cation with strong positive charge and PG is a negatively charged anion. When PL is administered intraabdominally it binds to the negatively charged injured peritoneal areas and forms a neutral matrix with the subsequently administered PG. The property of PL to migrate through the lipid bilayers[189] leads the PL/PG complex to further adhere to the wound. The polypeptide biofilm is thought to minimize the amount of fibrinous adhesion promoting exudates[190]. The PL/PG matrix is rapidly covered by mesothelial cells, documented with scanning electron microscopy (Fig. 4). In addition, the complex was shown to be biodegradable within 28 days and no side effects were noted on the function of peritoneal macrophages which are pivotal in the peritoneal healing process[186,187,191,192]. In addition, The PL/PG complex has no significant effect on other crucial key parameters in peritoneal healing such as tPA, PAI-1, TGFβ (transforming growth factor beta) and IL-6 (interleukin 6)[193,194].

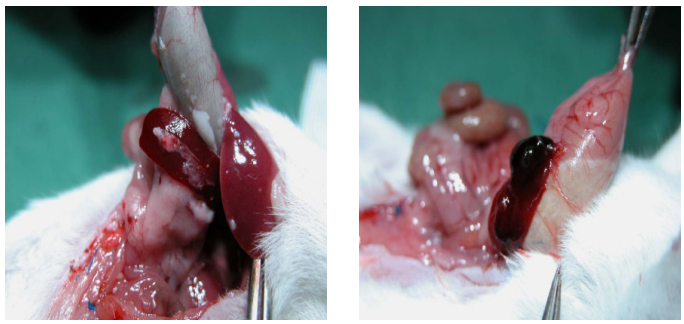
Figure 4. Electrone and fluorescence microscopy



Electrone microscopy showing smooth remesothelization, only leaving the outermost tip of the suture visible due to fixation artifacts (left). Fluorescence microscopy showing the polypeptide complex, green, covered with mesothelial cells, blue (right).

The PL/PG complex has shown to be effective in several different experimental settings, equivalent to concrete clinical situations. Tingstedt et al demonstrated a significant reduction of postoperative adhesions in a rat model where an ileocolic anastomosis was performed, under both clean and septic conditions. In addition the results indicated improved anastomosis safety with increased anastomosis burst pressure[195,196]. The administration of the differently charged polypeptides has also been shown to decrease postoperative bleeding and maintained adhesion reduction in mice where standardized wounds were induced to the liver and spleen (Fig. 5)[197]. Thoracic surgery is another surgical field where postoperative adhesion formation can cause difficulties when reoperations are required. The pleura is similar in structure as the peritoneum. Due to this the polypeptides were studied in a minor experimental pleural model and was shown to reduce adhesion formation[198].

Figure 5. Polypeptides in a model of bleeding



Mouse with a cut to the spleen, displaying the polylysine and polyglutamate complex covering the wound with no present signs of bleeding (left). Animal treated with saline, bleeding is present as a blood cloth (right). (Reprinted with permission from the publisher.)

The complex of PL/PG has not shown any toxic adverse effects. However administration of PL in high dose (200 mg/kg) alone, without the neutralizing PG, caused observed convulsions in the animals and subsequent rapid death[186]. The toxicity property of polycations is reported in the literature in different research fields. The mechanism of the toxicity is probably multi factorial and proposed explanations include direct cytotoxicity and apoptosis mediated via disruption in cell membranes[199], cell necrosis induced by tumor necrosis factor α[200], disruption of intracellular ion channels[201,202] and hemagglutination and hemolysis[203,204].

Our observation and the reported toxicity of polycations necessitate further research. Even though the PL is not intended to be used without the neutralizing anionic PG, it is of high importance that the individual ingoing substances are explored. PG has, in a previous study in the research group, not shown any toxic side effects in vivo or evidence of direct cytotoxicity in vitro when tested as single treatment[186]. There is support in the literature for the non toxic quality of PG[205]. Different molecular size of the PL as well as different chemical isoforms of PL can possibly influence both the toxicity profile and the anti adhesive effect in combination with PG. The isoform used in the former studies is α-poly-L-lysine (α-PL). Moreover, other polycations could possibly replace α-PL in the concept of oppositely charged polypeptides in postoperative adhesion prevention.

Aims

The aims of the individual studies were to determine…

I. early clinical and radiological parameters possibly predicting operative intervention for small bowel obstruction,

II. whether there is a difference in the frequency of readmissions due to small bowel obstruction after open versus laparoscopic surgery for suspected appendicitis,

III. the lowest effective dose of α-poly-L-lysine and poly-L-glutamate for postoperative adhesion prevention and to investigate the possible toxic levels of α-poly-L-lysine,

IV. the possible anti adhesive effect of another four cationic polypeptides and

V. the mechanism of in-vivo toxicity of polylysine and the biodistribution of polylysine and complex bound polylysine/polyglutamate when administered intraperitoneally as well as intravenously.

Material and methods

The different methods used are presented in even more detail in the individual scientific papers, appended at the end of the thesis. In this section there will be a somewhat more compromised presentation of material and methods. Table 1 show a short summary over the studies included.

Table 1. Overview of design and participants in the papers of the thesis.

	I	II	III	IV	V
Study design	Retrospective cohort study	Retrospective cohort study	Experimental animal study	Experimental animal study	Experimental animal study
Subjects	Humans	Humans	Mice	Mice	Rats
Method	Chart review	Chart review	Adhesion model	Adhesion Model	Toxicology/ Distribution
Number	109	4705	152	125	56
Male/ female	47/62	2461/2244	0/152	0/125	56/0

Retrospective studies

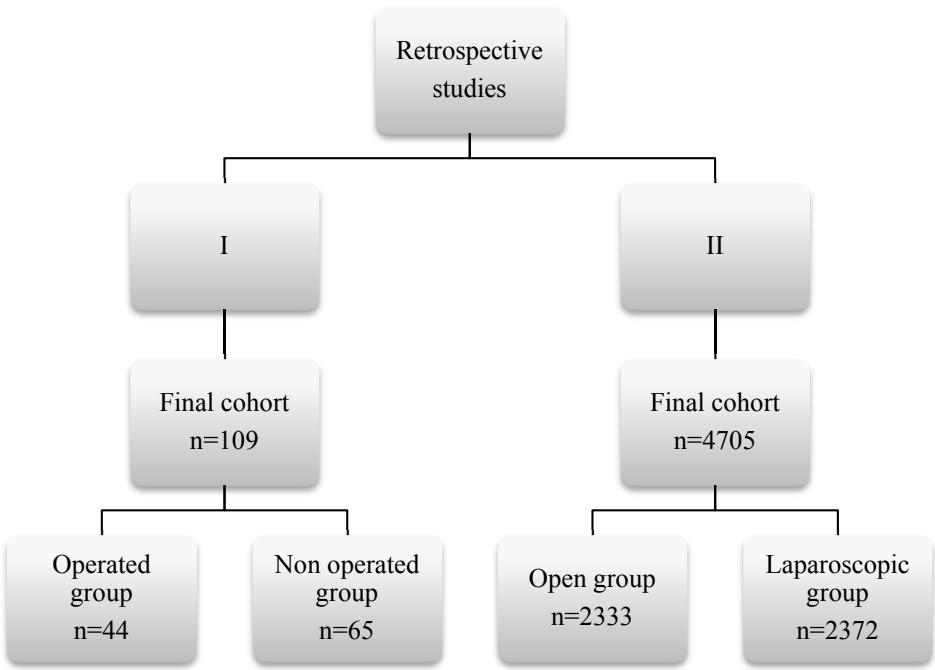
In 2011, the University Hospitals in Lund and Malmö were merged to form one university hospital, Skåne University Hospital. The hospitals are closely localized in the southern region of Sweden and provide acute health care for approximately 700 000 inhabitants together.

Paper I

Adult patients, 16 years or older, with the final diagnosis of small bowel obstruction and who had performed a follow-through examination at the Department of Surgery, University Hospital in Lund, between 2005 and 2006, were included in the study. Patients who had undergone abdominal surgery within 4 weeks before admission were excluded.

The medical record for each patient was reviewed for several laboratory and clinical parameters and the radiological examinations were examined for presence or absence of typical signs indicating high grade obstruction. In those patients who were surgically treated for their SBO episode, the cause of SBO and the surgical outcome was registered. The included patients were divided into two groups, one operated group (OG) where the patients had surgical treatment for their SBO and one non operated group (NOG) where the patients were conservatively managed. The distribution of included patients is depicted in Figure 6.

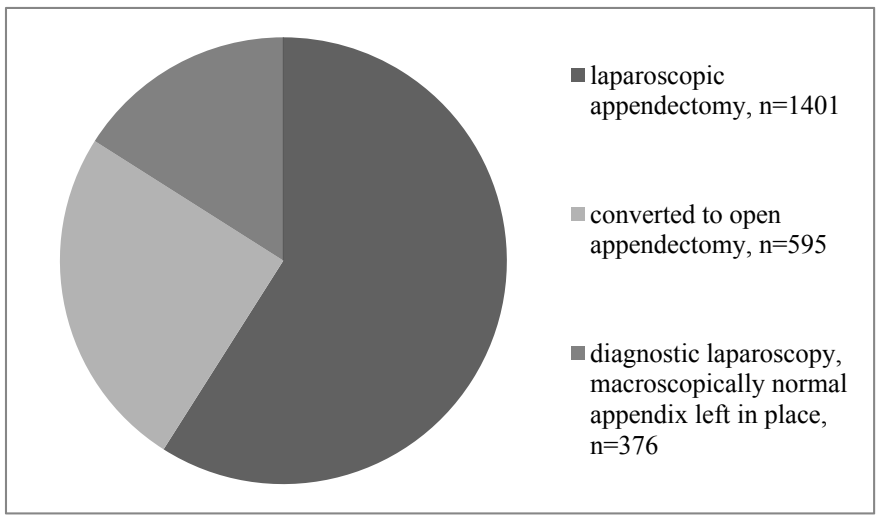
Figure 6. Distribution of patients in the clinical studies, I-II



Paper II

Data from patients, 16 years or older, operated on for suspected appendicitis between 1992 and 2007 at the University Hospitals in Lund and Malmö were retrospectively collected. During this time period the preferred operation technique in Lund was open approach and in Malmö laparoscopic operation was the procedure of choice. The open group (OG) consisted of all consecutive patients operated on with open technique in Lund and the laparoscopic group (LG) of all consecutive patients laparoscopically operated on in Malmö. The inclusion of patients is shown in Figure 6. The patients were identified by the ICD 9 and 10. In the OG an appendectomy was performed in all patients. The surgical outcome in the LG is summarized in Figure 7.

Figure 7. Surgical outcome in the laparoscopic group – paper II



Exclusion criteria were: incorrect operative code, incomplete or missing chart, extended surgery and cancer of the appendix. Patients in the LG who were randomized to open appendectomy after the diagnostic laparoscopy, due to an ongoing randomized study comparing open and laparoscopic surgery between March 2001 and July 2003[49], were also excluded. Moreover, patients readmitted for SBO with presence of adhesions already at the index operation, information of conflicting lower abdominal surgery or history of interfering disease (e.g. Crohn´s disease or abdominal cancer) were excluded.

All individual surgical charts were revised from the date of index surgery for suspected appendicitis until June 30, 2012. Patients who were readmitted and hospitalized for SBO had individual protocols established and data concerning gender, age, previous abdominal surgery, date of readmission, surgical technique at index operation and macroscopic appearance of the appendix were registered. The patients were analyzed

both on the intention-to-treat basis and per protocol. In the per protocol analysis the converted patients in the LG were excluded.

Experimental studies (III-V)

Animals

In study III and IV female NMRI mice, weight 25-30 g, and in study V male Sprague Dawley rats with an approximate weight of 250 g, were used. The animals were kept under standardized conditions with free access to tap water and pellets. The animals received pre- and postoperative standardized care in compliance with the guidelines of the Swedish Government and Lund University, Sweden. The studies were approved by the local ethical committee at the Lund University.

Chemicals

Osmotic balanced (2.54 wt % glycerol) aqueous solutions of α-poly-L-lysine (α-PL), poly-L-glutamate (PG), lactoferrin, lysozyme, polyarginine, ε-poly-L-lysine (ε-PL) and FITC labeled α-PL were freshly prepared on the day of the experiment. The ε-PL was purchased from Chisso Corporation (Tokyo, Japan). The other chemicals were all purchased from Sigma Aldrich (St Louis, Mo., USA). For histology and immunohistochemistry hematoxylin-eosin staining, FITC labeled CD31 and anti vWF were used (Abcam, Cambridge, UK).

Equipment

Surgical instruments (III-V), drapes, sponges (III-V) and capillary tubes (V) were retrieved from in vivo laboratory, BMC, Lund, Sweden. Peripheral venous catheters (V), needles and syringes (III-V) were purchased from Becton Dickinson, Helsingborg, Sweden). Monofilament suture (Prolene and PDS, Ethicon, Johnson & Johnson, Somersville, USA) was used for closure of peritoneal incisions and midline incision (III-IV). Obtained blood samples were analyzed using a blood-gas reader (Radiometer ABL 725, in vivo laboratory, BMC, Lund, Sweden) (V).

Anesthesia

Anesthesia was induced by an intramuscular injection of Ketamine (Ketalar, Pfizer, NY, USA) and Xylazine (Rompun Vet, Bayer AB, Gothenburg, Sweden). Ketamine 150 mg/kg and Xylazine 7,5 mg/kg was used in mice (III-IV) and the corresponding doses in rats were 60 mg/kg and 16 mg/kg, respectively (V). The animals were euthanized with an overdose of anesthetics while still under anesthesia at the end of the experiment. Buprenorphine was used for postoperative pain relief (III-IV).

Adhesion model – paper III-IV

In paper III and IV, adhesions were induced by a reproducible and standardized model, adopted from Holmdahl et al[206] and used in previous studies from our research group. After the abdomen was shaved and disinfected, a 25 mm long midline incision was made to enter the peritoneal cavity. On each side, parallel to the midline, a 15 mm long incision was created in the peritoneum of the abdominal wall. The incisions were closed by four interrupted sutures with sutures placed in both ends of the incision and the midline incision was closed by running suture in two layers with monofilament sutures.

Before abdominal closure the abdominal cavity was installed with different volumes and concentrations of the cationic polypeptides (α-PL, ε-PL, lactoferrin, lysozyme, polyarginine) shortly followed by installation of the anionic PG according to the experimental designs lined out in Tables 2-4. α-PL (III) and ε-PL (IV) were moreover installed as single treatment without the neutralizing PG to evaluate possible obvious toxic effect on the animals, the design for these parts are shown in the lower part of Table 2 (III) and in Table 5 (IV), respectively.

After one week, the animals were anesthetized and adhesions were evaluated. The abdomen was opened by a U shaped incision with its base on the right. The lengths of the previously created lesions as well as the lengths of the individual adhesions attached to the incision were measured with a caliper up to one-tenth of a millimeter. Data were expressed as percentage of the incision covered by adhesions; adhesions (%) = (sum of attachments in mm/incision length in mm) x 100. In the parts concerning toxicity, the animals were carefully and intensely observed for adverse reactions, impaired recovery or even premature death.

Table 2. Experimental design – paper III

Group	Animals (n)	Treatment	Concentration (mg/ml)	Volume (ml)	Dose (mg/kg)
Part 1					
1	10	PL+PG	5,0	1,0	200
2	9	PL+PG	1,0	1,0	40
3	10	PL+PG	0,5	1,0	20
4	10	PL+PG	0,1	1,0	4
5	9	PL+PG	0,04	1,0	1,6
6	10	PL+PG	0.01	1,0	0,4
7	10	Control (NaCl)		2,0	
8	10	PL+PG	5,0	0,1	20
9	10	PL+PG Local application	5,0	0,04	8
10	10	PL+PG Local application	1,0	0,02	0,8
11	10	Control (NaCl)		0,08	
Part 2					
12	12	PL	0,1	1,0	4
13	12	PL	0,5	1,0	20
14	12	PL	1,0	1,0	40
15	6	Control (NaCl)		1,0	

PL=α-polylysine, PG=polyglutamate, NaCl= sodium chloride. Volumes in the first part are per each substance, therefore, volumes of treatment substances PL+PG should be doubled.

Table 3. Experimental design – part one, paper IV

Group	Animals (n)	Treatment	Concentration (%)	Volume (ml)
1	9	ε-PL + PG	0.5 + 0.5	1 + 1
2	10	Lacto + PG	0.5 + 0.5	1 + 1
3	10	Lyso + PG	2.0 + 0.5	1 + 1
4	10	PA + PG	0.5 + 0.5	1 + 1
5	10	NaCl	0.9	2
6	10	α-PL+PG	0.5+0.5	1 + 1

ε-PL=ε-polylysine, PG=polyglutamate, Lacto=lactoferrin, Lyso=lysozyme, PA=polyarginine, α-PL =α-polylysine, NaCl=sodium chloride

Table 4. Experimental design – part two, paper IV

Group	Animals (n)	Treatment	Concentration (%)	Volume (ml)
1	10	ε-PL + PG	0.05 + 0.05	1 + 1
2	10	ε-PL + PG	0.01 + 0.01	1 + 1
3	9	ε-PL + PG	0.005 + 0.005	1 + 1
4	10	NaCl	0.9	2

ε-PL=ε-polylysine, PG=polyglutamate, NaCl=sodium chloride

Table 5. Experimental design – part three, paper IV

Group	Animals (n)	Treatment	Concentration (%)	Volume (ml)
1	5	ε-PL	0.5	1
2	5	ε-PL	0.1	1
3	5	ε-PL	0.05	1
4	5	ε-PL	0.01	1
5	7	NaCl	0.9	1

ε-PL=ε-polylysine, NaCl=sodium chloride

Toxicity and biodistribution model – paper V

The experiment was divided into two parts, one for toxicity and one for biodistribution and a schematic flowchart for the methods used are illustrated in Figure 8. After anesthesia was induced, intravenous access was established through the lateral tail vein by a peripheral venous catheter and intraperitoneal access was obtained through injection in the lower abdomen. Blood samples were collected from the orbital plexus.

In the part of toxicity the α-PL was administered in different body dose, as single treatment or with subsequent administration of PG, according to Table 6. The route of administration was either intravenous or intraperitoneal. Repeated blood samples were obtained for immediate blood gas analyses, as well as blood smears. Levels of hemoglobin, potassium, lactate, bicarbonate, base excess, pO_2 and pCO_2 were measured and the blood smears were conducted for erythrocyte evaluation. The time points for blood samples were 1, 5, 10, 30 and 60 minutes after administration of treatment substance. A blood sample and blood smear before administration of the treatment substance (time point 0) on each animal was taken as well and the baseline values of each animal and parameter were used as controls. Organs were harvested at

the end of the experiment to obtain tissue specimens for histology and immunohistochemistry.

In the second part of the study, the animals were given intravenous or intraperitoneal FITC labeled α-PL alone or in combination with PG (Tab. 6), to evaluate the accumulation in the circulation and the biodistribution to various organs. Blood samples were obtained at the same intervals as in part one to elucidate the levels of the polypeptides over time. Organs were harvested for fluorometric analysis.

Figure 8. Simplified schematic flowchart of the methods used in paper V

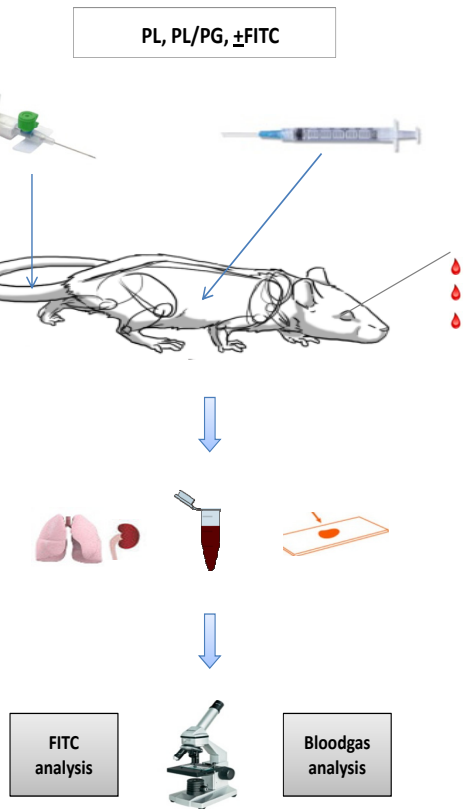


Table 6. Experimental design – paper V

Group	Treatment	Route	Body dose	Animals, n
1	PL	i.v	10 mg/kg	6
2	PL	i.p	10 mg/kg	6
3	PL Low	i.p	2 mg/kg	6
4	PL High	i.v	12.5 mg/kg	3
5	PL + PG	i.p	10 mg/kg	6
6	PL (Histology only)	i.v	15 mg/kg	2
7	FITC-PL	i.v	5 mg/kg	6
8	FITC-PL	i.p	5 mg/kg	6
9	FITC-PL + PG	i.v	5 mg/kg	6
10	FITC-PL + PG	i.p	5 mg/kg	6
11	NaCl (FITC-controls)	i.v	9 mg/ml	3

Body dose relates to the dose of administered PL, the dose of given PG was constantly 5 mg/kg. PL=α-poly-L-lysine, PG=poly-L-glutamate, FITC=fluorescein izothiocyanate, NaCl=sodium chloride, i.v=intravenous and i.p=intraperitoneal

Statistics

In paper I the Fisher´s exact test was used for univariate analysis to analyze differences for each predictive variable between the groups. Multivariate analysis was performed using logistic regression model to calculate for independent predictive factors.

In paper II the hypothesis was a lower frequency of SBO after laparoscopic surgery. A power calculation was performed and to prove a 50 % reduction of SBO in the laparoscopic group, a statistical power of 80 % and a risk of 5 % inaccuracy, a total of 1655 patients were needed in each group. Fisher's exact test was used to calculate for difference in the frequency of readmission for SBO between the two groups. Chi square test, Independent T test and Wilcoxon Rank Sum test were used to calculate differences

of gender, age and follow up time. Pearson's bivariate correlation and multivariate logistic regression analysis were used for calculation of possible independent risk factors for SBO.

In the experimental studies the numbers of animals in the groups were low and the distribution of the continuous variables were considered skewed and due to this, non-parametric rank sum tests were used to compare means between the groups. The Mann-Whitney U test was used to compare differences between individual groups and the Kruskal Wallis test was used to determine differences between several groups (III-V).

A p value < 0,05 was considered statistically significant. SPSS® (version 16,17 and 19) and SAS® (version 8.2) were used for statistical procedures.

Results

A summary of the results is presented in the following section and more detailed results are presented in the original communications.

Early parameters predicting surgery in patients with SBO -I

In paper I, we searched for early factors that possibly could predict surgical intervention in patients with SBO. 109 patients with final diagnosis of SBO and who had performed a follow through examination were included in the study and 44 patients had surgery and 65 patients were treated conservatively. We identified five parameters, clinical and radiological, that were significantly more common in the patients who were surgically treated for SBO. These parameters are presented in Table 7 and are all possible to obtain within four hours from hospital admittance.

Table 7. Frequency of the most significant factors

Parameter	**NOG (n)**	**OG (n)**	***p* value**
No previous surgery	1/65	9/44	<0.001
Dehydration	7/65	14/44	<0.01
CRP>10 mg/L (<4 h)	13/65	20/43	<0.005
No flatulence (24 h)	17/58	21/34	<0.005
Differentiated air-fluid levels	13/48	19/30	<0.005

NOG=non operated group, OG=operated group, CRP= C-reactive protein.

Absence of any factor favored a non-operative treatment, the presence of one parameter did not differ between the groups while two and three or more parameters favored operative treatment (Tab. 8).

Table 8. Association of significant parameters to surgery

Number of parameters	OG, n=44		NOG, n=65		*p* value
	Patients	%	Patients	%	
None present	6	13.6	30	46.1	<0.001*
One present	11	25.0	23	35.4	ns
Two present	14	31.8	10	15.4	<0.05**
Three or more present	13	29.5	2	3.1	<0.001**

No parameter present was significant in favour of the non operative treatment (*) .Two and three or more parameters were in favour of operative treatment (**).

All but one (1,5 %) in the group that were conservatively managed had no previous abdominal surgery performed in contrast to 9 (20,5 %) in the operated group. Lower abdominal surgery was the most common previous operation in both groups, 74 % in the non operated group and 59 % in the surgically treated group.

Adhesions were the cause of SBO in 59% of the cases that were surgically treated. 20/44 patients had ischemic signs intraoperatively. 10 of those had small bowel resection performed whereas the other 10 had transient ischemia that resolved in theatre.

Median time from hospital admission to start of operation was 60 hours (range 15-299). No difference in time to operation was seen between patients with intraoperatively ischemia and those without ischemia.

There was no mortality in either group. Median hospital stay was 13 days (range 3-44) in the operated group and two days (range 1-9) in the non operated group.

Incidence of SBO after surgery for suspected appendicitis - II

After exclusion (110 patients in the LG and 139 in the OG) 4705 patients that were operated on due to suspected appendicitis were included. 2372 patients were included in the laparoscopic group (LG) and 2333 in the open surgery group (OG). The mean follow up time was 133 months (SD=49) in the LG and 161 months (SD=53) in the OG (p<0,001). The LG consisted of 53% females and the corresponding number in

the OG was 43% (p<0,001). Mean age was 36 years (SD=16) in the LG and 35 years (SD=16) in the OG (p=0,072).

The incidence of SBO was low in both groups, 0,4 % (LG) versus 1,0 % (OG), the difference was statistically significant. Independent risk factors for SBO were age above median and open surgery (Tab. 9-10). The incidence of only late SBO did not differ significantly between the groups (Tab. 9). In the per protocol analysis, where the converted patients in the LG were excluded, the incidence of readmission for SBO in the LG was 0,3 % compared to 1 % in the OG (p=0,009).

Table 9. Patients readmitted for SBO

	Open group n (%)	**Laparoscopic group n (%)**	***p*-value**
Total patients	24 (1,0)	10 (0,4)	0.015
Late SBO only (*>30 days*)	16 (0,7)	7 (0,3)	0.061

Table 10. Analysis of patient factors on SBO outcome

	Univariate analyses		**Multivariate analyses**	
	p-value	**Odds Ratio (95 % CI)**	**p-value**	**Odds Ratio (95%CI)**
Female gender	0.606	1.25 (0.63-2.48)		
Age above median	**0.022**	**2.31 (1.16-4.63)**	**0.017**	**2.33 (1.16-4.67)**
Follow-up time above median	0.086	1.87 (0.92-3.79)		
Laparoscopic appendectomy	**0.015**	**0.46 (0.19-0.85)**	**0.016**	**0.41 (0.19-0.85)**

The characteristics for the patients that were readmitted for SBO are summarized in Table 11. Four of the ten patients that were readmitted in the LG were converted to open appendectomy and no readmissions for SBO were recorded for those who only had a diagnostic laparoscopy performed.

Table 11. Characteristics for patients readmitted for SBO after surgery for suspected appendicitis

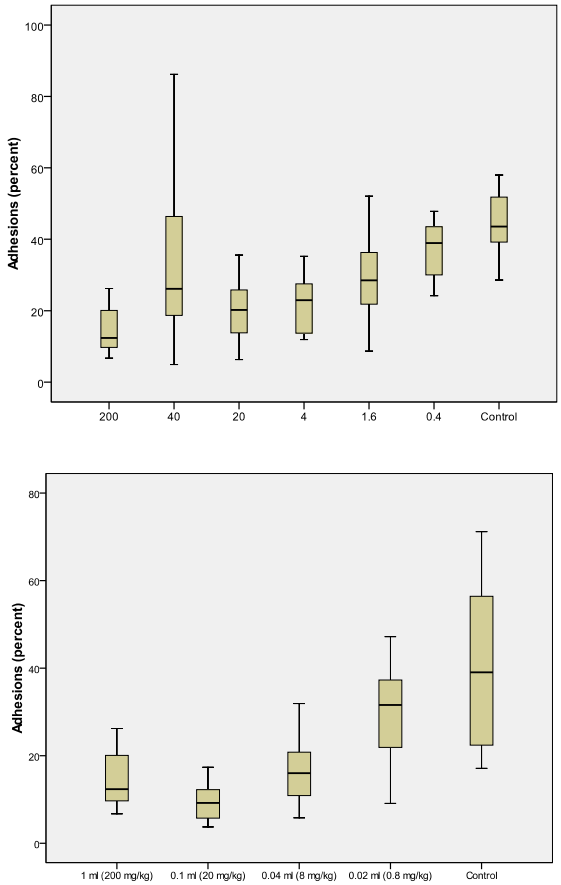
	Open group n=24	**Laparoscopic group** n=10
Status of appendix		
Perforated	10	4
Non perforated	11	6
Normal	3	0
Number of SBO episodes		
1	17	8
≥2	7	2
Total number for the group	39	14
Surgical treatment of SBO n (%)	13 (54)	5 (50)
1 operation	11	1
2 operations	2	4

Differently charged polypeptides – adhesion prevention, toxicity and biodistribution – III-V

Adhesion prevention

In paper III the anti adhesive effect of α-polylysine (α-PL) in combination with polyglutamate (PG) was dose dependent. The total dose of α-PL was altered by changing the concentration as well as by changing the volume. With decreasing dose the anti adhesive effect was decreased and the lowest dose with significant adhesion reduction was determined to 1,6 mg/kg (Fig. 9). All doses, except from the lowest concentration and volume, were significantly better than controls.

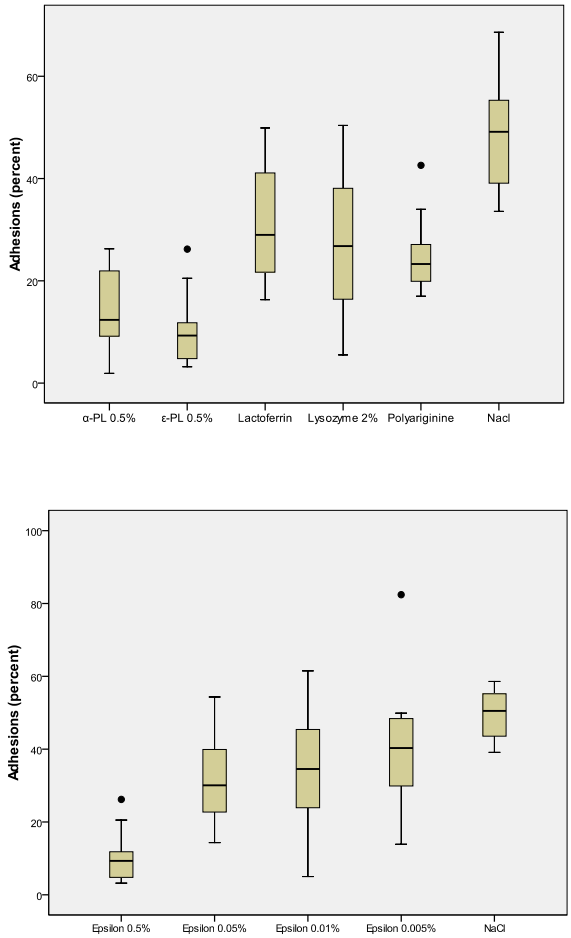
Figure 9. Adhesion reduction of α-PL in combination with PG



Adhesion reduction with different doses of α-PL by altering the concentration (top) or the volume (bottom). The doses on the x-asis is given in mg/kg (top) and in ml and mg/kg (bottom).

In paper IV ε-polylysine (ε-PL), polyarginin, lysozyme and lactoferrin in combination with PG were investigated. All four cationic polypeptides showed significant anti adhesive effect compared to controls. ε-PL was superior to the other three and was further evaluated at different doses. The ε-PL showed, as α-PL, a dose dependent response and the lowest significantly effective dose was 4 mg/kg (Fig. 10).

Figure 10. Adhesion reduction with various cations and ε-PL.



Adhesion reduction with various cations (top) and with different concentrations of ε-PL (bottom). The dots represent outliers.

Toxicity and biodistribution

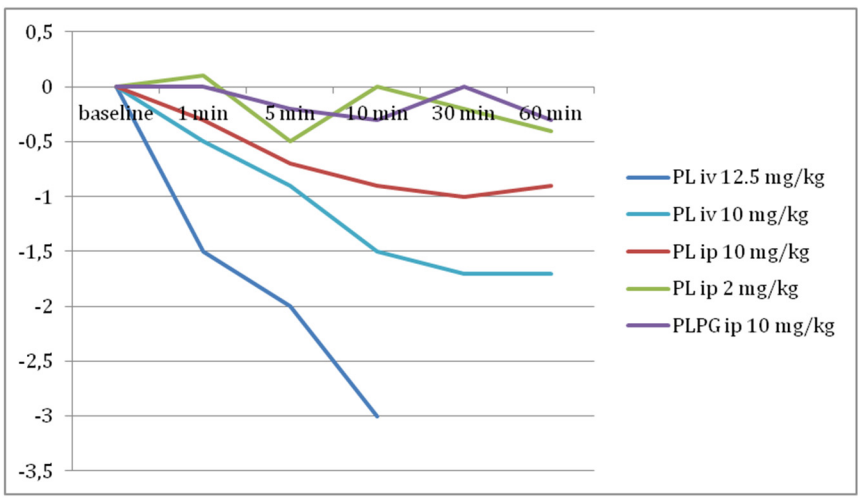
Single treatment with α-PL and ε-PL with the aspect of possible toxicity was studied in paper III and IV, respectively. As shown in Table 12, all animals survived and fared well in all doses except for six animals in the group that received α-PL and three in the ε-PL group. The LD50 was estimated to 40 mg/kg for α-PL and the corresponding dose for ε-PL was approximately 200 mg/kg. The experiments started with the lowest doses and no doses exceeding the lethal doses were tested.

Table 12. Outcome of toxicity test for single treatment with α-PL and ε-PL

Animals (n)	Treatment	Concentration (mg/ml)	Volume (ml)	Dose (mg/kg)	Survival (n)
	α				
12	α-PL	0,1	1,0	4	12
12	α-PL	0,5	1,0	20	12
12	α-PL	1,0	1,0	40	**6/12**
6	NaCl	9,0	1,0		6
	ε				
5	ε-PL	0,1	1,0	4	5
5	ε-PL	0,5	1,0	20	5
5	ε-PL	1,0	1,0	40	5
5	ε-PL	5,0	1,0	200	**2/5**
7	NaCl	9,0	1,0		7

In paper V we tried to further explore the toxicity and biodistribution of α-PL in an experimental setting in rats. In animals receiving high doses of α-PL alone intravenously a rapid decrease in PO_2 was registered, the decrease was statistically significant in the highest dose, 12,5 mg/kg. When α-PL was administered in high dose in combination with PG or as single low dose intraperitoneally there was no decrease in PO_2 observed during the experiment (Fig. 11).

Figure 11. PO_2 levels, diagram showing the mean difference in kPa (kilo Pascal) from baseline.



Edema was observed macrosopically when the lung was dissected in the animals receiving intravenous single treatment with high dose α-PL (Fig. 12). Histology revealed extravasated blood in lung and liver in the highest intravenous dose after 10 minutes (Fig. 13). Immunohistochemistry showed corresponding disturbed architecture of the endothelial lining.

Figure 12. Distended lung with extravasated blood (left) and edema seen in cross section of lung (right) in high dose single treatment with α-PL

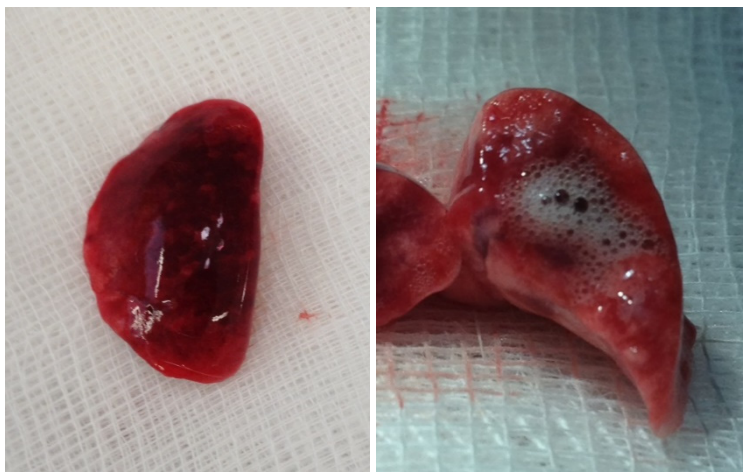
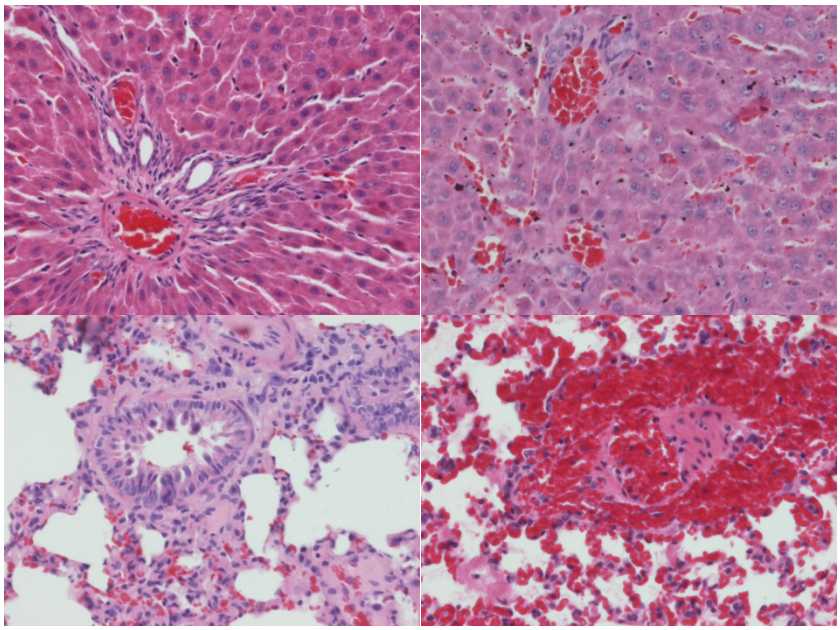


Figure 13. Histology from liver and lung in intravenous high dose and intraperitoneal low dose single treatment with α-PL.



Top left liver 2 mg/kg ip, top right liver 15 mg/kg iv, bottom left lung 2 mg/kg ip and bottom right lung 15 mg/kg iv.

The biodistribution was investigated using FITC labeled PL. FITC-PL reached high levels in blood and a rapid decline was observed for intravenous administration whereas the distribution to the blood was slow and low levels were observed when the route of administration was intraperitoneal. Subsequent administration of PG resulted in low levels irrespective route of administration (Fig. 14).

FITC analysis from harvested organs showed high accumulation in liver, kidneys and lungs after 30 minutes when PL was administered alone intravenously, the accumulation was lower when intraperitoneally installed. No increased fluorescent activity was detected in the examined organs when intraperitoneal administration of PL was followed by PG (Tab. 13).

Figure 14. Levels of FITC-PL in blood after single FITC-PL (top) and in combination with PG (bottom), i.v.=intravenous, i.p.=intraperitoneal

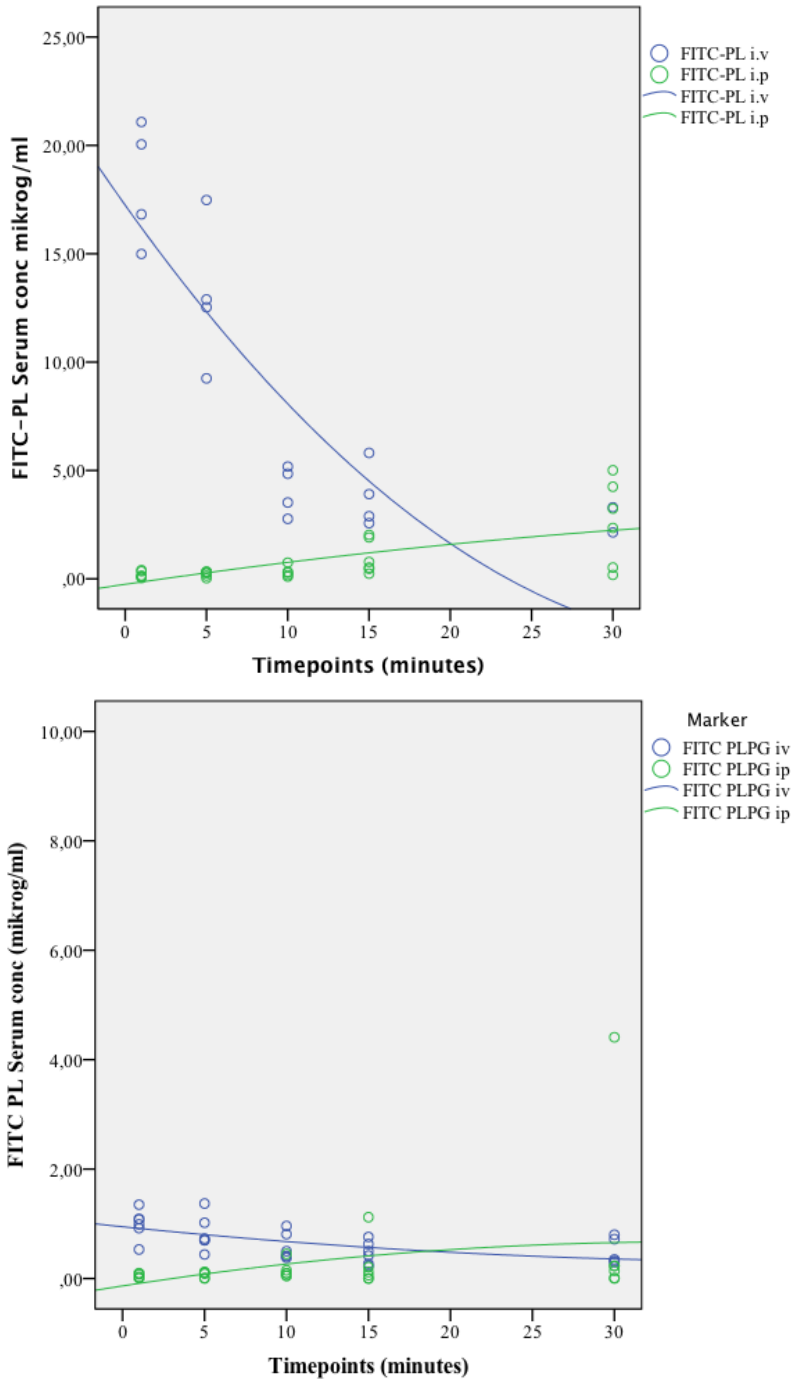


Table 13. FITC accumulation of FITC-PL in different organs 30 minutes after administration

Treatment / Organ	PL i.v.	PL i.p.	PLPG i.v.	PLPG i.p.	Controls
Kidney	+++	+	+++	0	0
Spleen	+	+	++	0	0
Lungs	+++	+	0	0	0
Liver	++++	0	+	0	0

Uptake measured as flourescens activity compared to controls. Mean values. 0 indicates 0-5 % increase, + indicates 5-25 % increase, ++ indicates 25-50 % increase, +++ indicates 50-75 % increase and ++++ indicates >75 % increase.

Discussion

Small bowel obstruction – surgical treatment and impact of surgical approach

Predictive parameters in SBO

We found that no previous abdominal surgery, CRP > 10 mg/L, no flatulence within 24 hours, dehydration and the presence of differentiated air fluid levels on the initial plain abdominal film were parameters more frequently observed in the operated group. The absence of any of these factors predicted a conservative treatment whilst the presence of two or more factors predicted surgical intervention. Identifying patients who need operative treatment for SBO and thereby also select those patients who can be managed conservatively is difficult and studies on early predictive parameters are scarce. The information of no previous surgery as a predictive parameter for operation has also been shown by Leung et al[35]. Postoperative adhesions are the most common cause of SBO and patients with no previous abdominal surgery are more prone to have other underlying etiologies and thereby do not profit from a conservative regimen to the same extent as patients with ASBO do. The finding in our study of an elevated CRP above 10 mg/L as a possible predictive factor for surgery is concurrent with Aldemir et al[207]. A rise in white blood cell count has been shown to predict operation and strangulation[207,208] as well as high fever and tachycardia[207]. In our study leukocytosis, fever and tachycardia were slightly over-represented in the operated group but did not reach statistical significance. However, a subanalysis was not conducted for the patients that had ischemia observed at surgery which could have been interesting. A rise in inflammatory response could reflect a more severe obstruction and an elevation of procalcitonin, another highly expedient inflammatory marker that is shown to indicate ischemic complications of SBO[36], further supports this theory.

An obstruction of the small bowel results in fluid retention within the bowel lumen proximal to the obstruction and gives rise to elevated pressure in the bowel wall. This in turn causes impaired venous backflow and lymph drainage and leads to accumulation of fluid within the bowel wall as well as intra-abdominally. Vomiting further exacerbates the fluid loss. The fluid loss would hypothetically be more pronounced when there is a high grade obstruction present. Dehydration was an independent risk factor for surgical intervention of SBO in our study. Differentiated air fluid levels

within the same dilated small bowel loop has been shown to indicate high grade obstruction most unlikely successfully treated non operatively [38]. The determination of its presence is done on an upright or lateral decubital position of the plain abdominal film, the latter position in our study. The use of regular or low dose CT as the initial radiological examination for diagnosis of SBO is more common nowadays due to the increased possibility to address the etiology of the SBO episode. However, plain abdominal film is still recommended to be the first choice of radiology in patients with ASBO without suspicion of strangulation[14] and therefore the presence of differentiated air fluid levels could be further examined in future studies validating the sign concerning prediction of surgical treatment in SBO. The aspects of radiation should also be considered when modality of radiology is chosen, especially in younger patients and patients with repeated episodes of ASBO for whom there is a risk of high accumulated radiation dose.

Adhesions were the etiology of the SBO in 59 % of the patients who had surgery in our study, which is in concordance with other studies[1,19,209]. Single band adhesion was the most common cause followed by multiple adhesions. The frequency of intraoperative ischemia was high in our study, 45 %, a high figure compared to other studies[1,19,210] and of those with ischemia, 50 % required small bowel resection. One possible explanation could be selection bias since only patients who had a follow-through examination performed were included in our study. Another plausible explanation could be a too conservative approach and delay in surgery. On the other hand, the time from admission to start of operation did not differ between those with ischemia and patients with no intestinal ischemia observed. Single band adhesions are reported to cause higher percentage of ischemia and single band adhesion was a frequent finding in our study[6,211].

The main purpose of the study was to identify early parameters that could predict operative intervention in patients with SBO, regardless of etiology, and thereby decrease the number of patients who will endure a failed small bowel follow-through examination. These factors could contribute to faster decision of surgery, reducing morbidity and hospital stay.

Open versus laparoscopic surgery for suspected appendicitis

In paper II we found a low frequency of readmissions for SBO in both groups, 1,0 % in the open group and 0,4 % in the laparoscopic group. The difference was minor though statistically significant. Approximately 50 % of the patients in both groups were operatively managed. Andersson et al reported a cumulative risk for surgically treated SBO after open appendectomy of 0,63 % after 1 year and 1,3 % after 30 years[45]. In the study by Tingstedt et al the incidence of assumed SBO after open appendectomy was 1,54 % during the follow-up time for approximately 10 years. In the same study they reported a risk for surgically treated SBO of 0,84 %[44]. Riber et al demonstrated an incidence of SBO requiring operative intervention of 0,79 % after 1 year and 1,51 %

after 14 years of follow-up[88]. The patients included in those studies underwent appendectomy between 1963 and 1993, 1981 and 1996, and 1978 and 1985, respectively, whereas the patients in our study had their index surgery between 1992 and 2007. The difference in time periods could possibly partly explain the lower readmission rate for SBO in our study. With time, surgeons have become more aware of the importance of atraumatic surgical approach, gentle tissue handling and hemostatic control. Starch powdered gloves, known to facilitate adhesion formation and thereby contributing to postoperative SBO, are no longer in use. The long follow-up time in the study by Andersson et al should also be taken in to consideration since the risk of postoperative SBO is lifelong.

Comparison with other studies concerning SBO after laparoscopic versus open surgery for appendicitis is complicated due to heterogeneity of the study designs. The incidence of SBO has been reported lower in laparoscopic approach in children[92,93]. Angenete et al demonstrated a reduction in the incidence of SBO following laparoscopic appendectomy compared to open appendectomy, though the cohort included children[95]. Masoomi et al showed that approximately 70 % of all surgery for suspected appendicitis in 2008 in adults was conducted by laparoscopic approach and found a significant difference in postoperative bowel obstruction for uncomplicated as well as complicated appendicitis favoring the laparoscopic group. However, in that study they were not able to provide the conversion rate and no information on postoperative complications arising after discharge which is a limitation[84]. Other studies, including systematic reviews, could not show any differences in SBO between the surgical approaches[98,212,213]. In the study from Leung et al they could not show a statistically significant difference between open and laparoscopic appendectomy[47]. In a commentary on Leung's study, O'Connor et al proposed the risk for a type II error due to that only one third of the patients had laparoscopic surgery performed[214]. The design in our study was intention-to-treat, thereby including all possible surgical outcomes in the laparoscopic group. The inclusion of diagnostic laparoscopy for suspected appendicitis rendering in leaving a macroscopically normal appendix in place, probably partly explains the low readmission rate in the laparoscopic group.

Open appendectomy and age above median turned out to be independent risk factors for development of SBO. Age has been reported as a risk factor in other studies [45,95,212]. Andersson demonstrated that age above 70 was accompanied with the highest risk and age between 20 and 39 having the lowest risk[45]. The SCAR-3 study reported an increased risk for readmissions directly related to adhesions in patients aged 16 or more[212].

The distribution of gender and age were in concordance with other studies[67,84,96]. There were more females in the laparoscopic group compared to the open group. Female gender has been demonstrated to be associated with increased risk for SBO after appendectomy[88] as well as decreased risk[45]. In our study, female gender did not turn out to be a risk factor.

The mean follow up time was 13,5 years for the open group and 11 years for the laparoscopic group. The follow up time is one of the longest regarding SBO after surgery for suspected appendicitis.

The overall conversion rate in the present study was high (25 %). When appendicitis was diagnosed at laparoscopy only the surgeons that had accreditation for laparoscopic appendectomy progressed with a laparoscopic procedure and converted in case of technical difficulties, while surgeons lacking accreditation converted to open appendectomy after the diagnostic laparoscopy. The conversion rate diminished by time as the educational program proceeded. Conversion rate is in the literature reported from 5,8 to 28 %[49,215-217].

Patients who undergo appendectomy have comparatively low risk of readmission directly related to adhesions, a statement made by Parker et al in the SCAR-3 study[212]. This is the latest of three epidemiological studies using the Scottish National Health Services medical record linkage database to assess the extent of adhesion related readmissions after lower abdominal surgery. The overall readmission rate in SCAR-3 study was 0,9 %, however the number of appendectomies performed accounted for approximately 30 % of all abdominal surgical procedures and 7 % of all patient readmissions during a 5 year follow up. Due to this, appendectomy contributed significantly to the overall burden of adhesion related readmissions. In a study from Sweden late readmissions occurred in 2,94 % after open appendectomy with a median follow-up time of 10 years and the risk for readmission was highest for those patients with complicated appendicitis or negative appendectomy[44]. Ditzel et al could not find any influence of surgical approach or whether the appendicitis was advanced or not in the incidence of abdominal complaints after appendectomy[218].

Methodological considerations (I-II)

Retrospective studies are generally considered inferior to prospective study design. Some investigators imply that retrospective studies are "quick and dirty" because the data are quickly collected from records to answer a question and propose that retrospective studies should not be performed when a prospective study design is feasible. The disadvantages of retrospective studies are, among others, the lack of randomization and the risk of missing data. Although retrospective studies are quicker to conduct than prospective studies and afflicted with lower grade of scientific evidence, they can, if properly performed, often serve as a first survey and give an opportunity to design an appropriate and adequate hypothesis in a following prospective study. With this in mind, conclusions from retrospective studies should be drawn with humbleness and carefulness.

In paper *I* the fact that not all patients had a plain abdominal film performed, due to an ongoing study concerning low dose CT, resulted in that the sign of differentiated air fluid levels could not be determined in those patients randomized to low dose CT.

The sign of differentiated air fluid levels within the same dilated small bowel loop is retrieved from a supine or lateral decubital position, not undertaken when low dose CT is chosen as initial radiological examination. However, the majority of the patients in both groups had plain abdominal films done and the frequency of patients lacking a plain film was equal in the groups. Absence or presence of flatulence within 24 hours is another parameter where information was missing in some patients due to that the information was not possible to retrieve from the chart or because the question was never asked to the patient. These issues could have been avoided or at least diminished in a prospective design with a specific protocol.

The definition of dehydration in our study could be regarded as indistinct. The truly presence or absence of dehydration in the adult patient is not always easy to determine. The lack of standardized clinical methods for assessment of dehydration is a limitation. To confirm dehydration the most accepted process is to calculate the fluid loss by weight change given as percentage of total body weight[219]. This assessment requires a current reliable weight pre hospitalization which would probably be missing in a great number of adult patients. The determination of dehydration by clinical assessment for mucosal dryness, reduced skin turgor, decreased urinary output, vomiting, hypotension etc, in combination with laboratory parameters is commonly used. However, it could be a vulnerable method especially in a retrospective study design.

In paper *II* there are also methodological issues to consider. The inclusion of two hospitals with different surgical approach is a limitation. As stated previously the two hospitals are closely located and merged to one University Hospital since 2011, however the fact that more surgeons are involved remains. The strict educational program for accreditation before being allowed to do laparoscopic appendectomy in Malmö resulted in high conversion rate as stated above. You may object to whether these patients that were converted due to lack of accreditation should be included at all. On the other hand, the program led to high competence in laparoscopic appendectomy. Lack of competence of a newly adopted surgical technique could otherwise be a contributing factor to increased patient morbidity, not observed in the present study.

When comparing postoperative complications, including SBO, and mortality after open and laparoscopic appendectomy, converted patients are not seldom transferred to the open appendectomy cohort[86]. This was not done in our study due to the fact that conversion is one of the three surgical outcomes in the laparoscopic approach for suspected appendicitis. Furthermore, the open group did not include any patients that had initial laparoscopy preceding the open appendectomy. Another issue to discuss is the inclusion of SBO within 30 days and this is the reason why we extracted the patients with only late SBO in Table 9, in the overview, to visualize the distribution. Early SBO after surgery is a specific entity and could sometimes be hard to distinguish from postoperative ileus. However, if typical symptoms and positive radiologic findings are present, the majority of early postoperative SBO episodes are thought to be mechanical and probably caused by adhesions and rarely due to paralytic ileus[90,220]. Stewart et al

showed that the risk for early SBO is increased for operations performed below the transverse mesocolon[91]. 0,31 % of the patients were readmitted for SBO within 4 weeks after open appendectomy in the study by Tingstedt et al where 50 % were surgically treated[44]. Andersson et al reported a cumulative risk of surgically treated SBO to 0,41 % after four weeks[45]. Whether patients with early SBO are included or not should be considered when comparing studies.

There is a risk of missing patients being readmitted for SBO in other hospitals or other clinics since only the surgical charts for the hospital where the patient had his or her index operation were reviewed. However, we calculate the possible loss of patients to be fairly equal in both groups.

Despite the retrospective design of this study the large and equal number of patients in the groups must be a strength as well as the manual revision of the charts, thereby increasing the possibility for correct coding of symptom diagnoses and operation codes. It has been shown that only approximately one third of the patients were correctly coded in a study of the natural history of ASBO[221]. The inclusion by intention-to-treat, including all possible outcomes in the laparoscopic group, is for sure confusing for many but we think that it rendered in a just comparison of the two mainly used surgical approaches for suspected appendicitis.

Differently charged polypeptides-adhesion prevention, toxicity and biodistribution

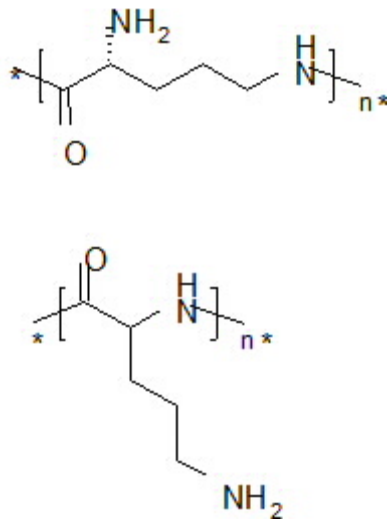
Adhesion prevention

In the first experimental study we could reproduce the previously shown promising adhesion prevention of the combination of the cationic α-PL and anionic PG. The adhesion preventive effect of α-PL/PG was proved to be dependent of dose. Lowering the total dose either by altering the volume or the concentration resulted in decreased effect. The lowest effective dose of α-PL was determined to 1,6 mg/kg. The observed toxicity of single treatment with α-PL was established to 40 mg/kg intraperitoneally. The gap between the lowest effective dose and the toxic dose was considered too narrow. Therefore, we proceeded to investigate the effect of ε-PL, polyarginine, lysozyme and lactoferrin in combination with PG. All four resulted in decreased adhesion formation when compared to controls, ε-PL being superior to the others and was furthermore studied for different doses. As α-PL/PG, the effect of ε-PL/PG showed a dose dependent pattern and was afflicted with lower toxicity than α-PL when administered alone.

Polypeptides are a group of macromolecules used widely in biological research in different areas such as chemotherapeutic drug carriers, gene vectors, coating of

implants, antimicrobial and antineoplastic research. They are water-soluble, biodegradable and often described as non toxic for humans and the environment[222]. In the previous studies from the research group and in third paper of this thesis, the cationic polypeptide used was α-poly-L-lysine (α-PL). The α-PL is a long helix with long side chains that elongates when in contact with the cell membrane. ε-poly-L-lysine (ε-PL) is another isoform of PL which also has a linear structure, though shorter and with shorter side chains. It is naturally occurring and is secreted by various Streptomycetaceae bacteria and some filamentous fungi. It has the capacity of inhibiting growth of both grampositive and gramnegative bacteria and the molecule has been utilized for many years as a food preservative in Japan[223,224] (Fig. 15).

Figure 15. Chemical structure of ε-poly-L-lysine (top) and α-poly-L-lysine (bottom), the monomer defined within square brackets.



Polyarginine is a well-known linear protein transduction domain used to transport molecules into cells[225] with approximately the same size as α-PL. The two other cations studied were of globular structure, i.e. lysozyme and lactoferrin. Lysozyme is a small enzyme that is part of the immune system and lactoferrin, also known as lactotransferrin, is a functional glycoprotein containing many polycation domains. Both are found in mucosal secretions such as tears and saliva and have anti inflammatory and anti microbial activity[226].

The findings in paper III and IV indicates that a polypeptide with linear structure has better adhesion preventive effect than polypeptides of globular structure, probably due to the fact that the globular polypeptides have most of their positive charge turned

inwards. The cationic polypeptides with linear structure that expose their positive charge are thought to facilitate the binding and anchoring to the negatively charged damaged peritoneum.

Toxicity and biodistribution

Even though several reports have stated the non-toxic qualities of polypeptides there are also the reverse reported for cationic polypeptides when used in vitro and in vivo[199,203,227,228]. The cationic polypeptides in our concept of differently charged polypeptides in adhesion prevention are not effective as single treatment and are not supposed to be used without the neutralizing polyglutamate and there has been no toxicity observed for the complex of PL/PG. However, the individual substances must be safe to use and thereby the possible toxicity of α-PL, and subsequently the almost equally effective ε-PL, was investigated in the mouse model (paper III-IV). The approximated intraperitoneal LD50 for α-PL was estimated to 40 mg/kg and 200 mg/kg for ε-PL. The less toxicity observed with ε-PL is thought to be due to its smaller size than α-PL. The molecular weight for α-PL and ε-PL used in our study is > 30 kDa and 4,7 kDa, respectively. A previous study on the anti neoplastic capacity and toxicity afflicted with poly-L-lysine has demonstrated a cumulative intraperitoneal LD50 well in line with our result and the same study confirmed that the toxicity was related to molecular weight, increasing size rendered in increased toxicity[229]. The side chains on ε-PL is shorter than on α-PL which could be another hypothesis for lower toxicity, the shorter side chains may generate less or more superficial interaction with the lipid bilayer. In vitro studies on mesothelial cell lines showed high impact of linear polycations with high molecular weight with subsequent low proliferation rate of the mesothelial cells. There was little or no disturbance of the proliferation rate observed with ε-PL, polycations with globular structure or when the high molecular α-PL was combined with PG (data not published).

To further explore the toxicity afflicted with α-PL we tried to clarify the mechanism of the toxicity and moreover investigate the biodistribution of the polypeptide as single treatment or in combination with PG (paper V). We found a possible clarifying mechanism for the toxicity mediated by damage of the endothelial lining. The histology revealed extravasated blood in lung and liver in intravenous high dose single PL treatment. In the lungs macroscopically sanguineous edema was observed. When administered intraperitoneally the dynamic is thought to be similar but slower and could explain the lower toxicity. There is support in the literature for our findings of PL causing endothelial cell damage. Morgan et al suggest that PL is distributed to different organs and exerts cell membrane toxicity in the capillaries rendering in leakage through the endothelial lining [227]. Elferink showed that polylysine cause a time and concentration dependent lysis of polymorphonuclear leukocytes and that the lysis was annihilated with the administration of the polyanion polyglutamate[230]. Further he proposes that the positive charges have to be present on a polymere molecule of

sufficient length and that the interaction is most likely to occur with the phospholipids in the plasma membrane. The neutralizing effect of polyglutamate is supported by our present and previous studies whereas the toxicity is only proven with single administration of polylysine and the toxicity is low with low concentration. DeVries et al gives further support to our observations of edema and the anionic antagonistic effect of PG[231]. They showed remarkable distention and edema of the lungs in rats given lethal doses of polylysine intravenously. Moreover, they demonstrated an inhibition of the fatal toxicity when simultaneous or delayed administration of heparin or poly-L-aspartic acid was done. Heparin as well as poly-L-aspartic acid are strong anionic molecules.

Previous research has mostly focused on intravenous administration and the mechanism of toxicity has been reported to be due to hemagglutination and lysis of red blood cells[203], something that we could not demonstrate in the present study. The blood smears after intravenous as well as intraperitoneal administration did not reveal any differences from baseline sample before administration regarding hemagglutination or lysed cells. PG has been proven to be atoxic and its administration reduces toxic effects assigned with chemotherapy[184].

The doses of polylysine used in the first part of the fifth paper, i.e. the toxicology part, were based on the literature and the results from paper III. The LD50 for high molecular weight PL (>30kDa) is reported to be 12-15 mg/kg when administered intravenously[203] and we estimated the corresponding LD50 for intraperitoneal administration to 40 mg/kg. The reason why we in addition chose to administrate 2 mg/kg, i.e. just above the lowest effective dose for adhesion reduction (paper III), via the intraperitoneal route, was to prove that no adverse reactions were accompanied with the dose accepted for further studies. With this low dose there were no significant alterations in blood gas parameters and no pathological changes observed on histology.

The distribution of intravenously and intraperitoneally administered PL was investigated by measuring the levels of FITC labeled PL in blood as well as harvested whole organs. Intravenous administration resulted in a quick accumulation in the blood and rapid clearance as well. The intraperitoneal route rendered in a slower distribution pattern. The time points were based on the knowledge from the literature of rapid clearance of PL from the blood and this is probably the explanation why we did not catch the peak or identified the time point for decline for the intraperitoneal route. The distribution and accumulation in different organs after 30 minutes reflect the same fashion with lesser uptake when given intraperitoneally. When adding PG, the complex is neutralized in charge and the affinity to serum proteins is lowered. This probably explains the lower levels in blood and organs.

The magnitude and clinical burden of postoperative adhesions is substantial as depicted earlier. However, the conditions caused by adhesions are benign and any preventive agent has to fulfill a number of important criteria including a non toxic quality. Previous studies from the group have shown that the PL/PG complex can be used safely

in different clinically relevant situations such as the presence of a bowel anastomosis, septic condition or inadequate hemostasis with preserved effectiveness[195-197]. In addition, there has been no negative impact on peritoneal macrophages or key parameters of the peritoneal healing process[187,193,232]. The anti adhesive effect as well as the toxicity observed with single administration of polylysine is, together with supporting findings in the literature, most certainly dependent of size, configuration and charge density of the polycation used[230]. It is a delicate balance to find the sufficiently long and charged polycation that, in combination with polyglutamate, reduces postoperative adhesions to a sufficient extent without exerting any possible toxic side effects. However, most of the complex bound PL/PG is not transported to the blood circulation within 30 minutes after intraperitoneal administration and is most probably degraded in the abdominal cavity.

Moreover, when studying prevention of postoperative adhesions you have to be provocative to yourself and consider the actual fact that even if you can prove a significant reduction of adhesions, it is not equivalent to a prompt reduction of the clinical adhesion related complications such as SBO, infertility and abdominal pain. The anti adhesive agents approved for clinical use today have until today not clearly shown reduction in these hard endpoints[162,233]. The presence of a hard single band adhesion can cause more harm than multiple filmy adhesions. The risk of endured operation time and inadvertent bowel injury due to extensive postoperative adhesions would though be lowered even if the adhesion formation is reduced but this is not elucidated.

Methodological considerations (III-V)

The surgical adhesion model used in study III and IV is a model developed by Holmdahl et al[206]. The model is quite easy to perform and the procedure is standardized and reproducible. The choice of model was based on the previous studies in the research group as well as the time point for evaluation of adhesions, i.e. one week after surgery [186,187,234]. The evaluation of adhesions by measuring the length of adhesions to and in relation to the length of the lateral abdominal wounds with a caliper has its limitations. It doesn't tell us anything about the quality of the adhesions, something that is thought to play a significant role in a clinical situation. The evaluation of postoperative adhesions in experimental settings is often divergent and is influenced by the surgical model used for adhesion induction. There are several systems developed for scoring and evaluation of adhesions. Adhesions could be evaluated concerning quantity, by actual numbers or percentage of a depicted area, or be scored according to quality, e.g. thickness, tenacity and presence of vascularization. There is a diversity of scoring systems available including Nair[235], Bothin[236], Diamond[237], Zuhlke[238], Oncel[239] and Lang[240]. This unveils the actual problem of interpreting and comparing reports. This is also a problem in the real clinical situation where the evaluation of postsurgical adhesions in patients does not depend on any unified scoring system, if at all scored.

There is a call for uniformity of measurement and evaluation of adhesions and there is a recent proposal given for the use of a score called PAI, peritoneal adhesion index[14,241]. A standardized easy and user-friendly scoring system for evaluation would also facilitate and enable a more correct comparison of clinical studies concerning postoperative adhesion formation. If there was any hidden motive when deciding the name of the score is not revealed, however, having the abbreviation for the crucial restrictor of fibrinolysis in mind, PAI, the name must be questioned.

In paper III and IV, the aim of using glycerol water in our experiments was to osmotically balance our solutions. Previous experiments have been undertaken within the research group that has excluded an anti adhesive effect of the glycerol water per se (data not published). The controls received sodium chloride in corresponding volumes, an alternative could have been no installation at all in the control animals. However, you could not exclude a possible effect by hydroflotation in addition to the barrier film created by the complex of PL/PG, chiefly in the animals receiving the highest volumes. There has moreover recently been shown an anti adhesive effect of intraperitoneal administration of cold saline in an experimental setting[242].

In paper III and IV the possible toxicity of α-PL and ε-PL was investigated by observing the animals concerning behavior, recovery and possible death. You may object to this strategy and regard the evaluation as arbitrary and subjective. LD 50 is the median lethal dose or the amount of the substance causing the death of 50 % of a group of test animals. LD50 studies are rare nowadays and being phased out and replaced by less lethal studies. Our intention was not to conduct a regular LD50 toxicity study and there were no further doses given above the dose resulting in death and therefore the LD50 was estimated and not statistically calculated. The numbers of animals in paper IV concerning the toxicity of ε-PL was reduced for ethical aspects and therefore the approximated LD50 is even more speculative.

The method of sampling blood from the orbital plexus applied in paper V is quite delicate and deserves a comment. The blood is collected by thin glass capillary tubes utilizing the capillary pressure. The technique is somewhat tricky. Repeated blood sampling from the orbital plexus is accompanied with significant risk of visual impairment and is hardly or not maintainable from an ethical aspect when the animals are supposed to wake up after the experiment. The animals in our study were all determined at the end of the experiment, at the latest at approximately 60 minutes from the first collection of blood.

Conclusions

I. No previous abdominal surgery, CRP > 10mg/L, no flatulence within 24 hours, dehydration and presence of differentiated air fluid levels on plain abdominal film are five parameters that predicted surgical treatment of SBO in our study. These parameters are all possible to achieve within four hours from admission and may be helpful to advance the selection of patients for operative intervention.

II. The incidence of SBO after surgery for suspected appendicitis was low in open as well as laparoscopic surgical approach, a minor but still significant difference was demonstrated favoring the laparoscopic approach. High age and open surgery were identified as independent risk factors for subsequent SBO.

III. The adhesion preventive effect of PL/PG is dose dependent. The lowest effective dose of α-PL would probably be too close to toxic dose in a clinical situation even though no toxicity is observed of the PL/PG complex.

IV. All four alternative cationic polypeptides showed anti adhesive effect. The superior ε-PL showed a dose dependent pattern and its toxicity was lower compared to α-PL. The ε-PL could be an alternative in replacement of the α-isoform in combination with PG in future studies.

V. Injury to the endothelial cells with subsequent extravasation of blood and edema is thought to be one of the underlying mechanisms for the toxicity of PL. The biodistribution and accumulation of PL and PL/PG in blood and organs is slower and lower after intraperitoneal installation compared to intravenous administration where the peak as well as the clearance of the substance is rapid.

Future aspects

Worldwide, bowel obstruction and appendicitis are, together with incarcerated or strangulated hernias, volvulus and acute biliary disease, the most common causes of acute abdomen in adults. The major cause of SBO in high income countries has been postoperative adhesions since decades, while the etiological panorama is different in low and middle income countries. However, there has been a change and adhesions are becoming more common as a result of rising number of abdominal surgeries performed in those countries. The rates of major and emergency surgical operations are differing widely in the world due to the wide variability in capacity of providing appropriate health care to patients. The USA has 9 general surgeons per 100 000 inhabitants compared to less than 1 per 100 000 in many African countries[243]. In developing countries the pathologies needing surgery are present but the restrictions of available qualified professionals, equipment and health care accommodations result in lower frequencies of surgery. Even though the capacity of surgical care in many low income countries is restricted, there is a gradual success in development and the numbers of general surgeries performed worldwide will rise with time. Laparoscopic surgery has gradually replaced open surgery as the primary choice of operative approach for many general surgical conditions. The less traumatic surgical technique used today and the atraumatic laparoscopic approach is shown to decrease adhesive related complications including ASBO[95,209,213,244]. However, with increasing global number of abdominal operations the clinical burden of adhesions will most probably not decrease. Abdominal adhesions are generally not considered a "hot topic", although being the most common postoperative complication the knowledge and awareness of adhesions and the accompanying clinical complications must be alerted. Moreover, the research for advanced and correct selection of patients needing emergency surgery and providing more evidence for clinically significant advantages of certain surgical approaches for different diagnoses must go in parallel with further research for safe and clinically effective anti adhesive agents.

The results from the studies within this thesis generated thoughts and ideas that ought to be further evaluated.

1. To have supporting early predictive parameters to select patients with SBO for surgery or conservative management would benefit both patients and the surgeons. The early factors favoring surgical treatment for SBO in our retrospective study need to be further explored. A prospective design is preferred to validate the parameters. The parameters included in the protocol must be well defined. Dehydration was one of the

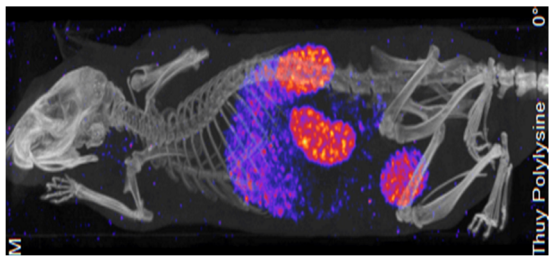
independent risk factors for surgery in our study and must be more strictly defined in a prospective setup. The fact that the use of plain abdominal film has been more or less replaced by low dose or regular CT is another issue to consider whereas the presence of differentiated air fluid levels is determined on a plain abdominal film.

2. To confirm the advantages of laparoscopic surgery for suspected appendicitis concerning SBO in an adult cohort, a prospective randomized study with long-term follow-up would be appropriate. Due to a low incidence of postoperative SBO in either surgical approach, the number of patients included in such a study has to be large. It would be difficult to conduct at a single center. Another issue could actually be to include patients. The laparoscopic approach is already established and has more or less ruled out open appendectomy in some hospitals.

3. The need for an effective anti adhesive agent that could significantly reduce the frequencies of adhesion related complications is huge. The administration of differently charged polypeptides establishes an interaction with damaged peritoneum resulting in a protective barrier film reducing the formation of postoperative adhesions. Whether the PL/PG complex has a final effect on subsequent SBO, infertility and abdominal pain is yet to be determined. Colorectal surgery is a leading cause of postsurgical adhesions[5,25,98,245,246]. Suitable patients to include in an initial pilot study could be those who will undergo colorectal surgery with a temporary ileostomy planned for secondary surgery. To progress with a clinical study on humans there are some obstacles to pass.

4. The toxicity observed for single use of PL, in particular the most effective α-isoform, is a problem. Even though the neutralizing PG is always intended to be added, the safety for the ingoing substances is of outermost importance. The span between the lowest effective dose and toxic dose is 20-fold, however this could be too close. A chemical alteration of the cationic polypeptide to reduce charge density or spatial properties have been discussed as well as construction of a premix of PL/PG, the latter did not succeed due to precipitation of the solution. The less toxic ε-PL will be further investigated and the biodistribution of PL and PL/PG will be completed with SPECT-CT. The imaging is performed after intravenous and intraperitoneal administration of PL and PG labeled with isotope. Figure 16 shows SPECT-CT of a pilot animal 1 hour after intravenous single treatment of PL, revealing low uptake in the liver and high accumulation in the kidneys and urinary bladder.

Figure 16. SPECT-CT, biodistribution of α-PL, 1 hour after iv administration



Lund University Bioimaging Center, LBIC, is greatly acknowleged for providing experimental resources.

5. The possibility to use PL/PG in different clinical situations with persistent anti adhesive effect has been shown as previously described. Further development of a spray application has recently been evaluated with success in a dose of 2 mg/kg[194]. The method of spray application would be feasible regardless of operative approach. Even more optimal spray devices must be developed, securing the obligate sequential administration of both polypeptides.

6. Furthermore, a substantial number of abdominal and pelvic surgeries are due to malignancies and it would be devastating if an anti adhesive agent promoted cancer cell growth. A pilot study from our research group demonstrated reduced cancer cell attachment in vitro and a significant reduction in tumor growth at surgical trauma sites in a rat model where the animals were inoculated with colon carcinoma cells before treatment with the bioactive polypeptides α-PL/PG (data not published). These results will be further evaluated in an extended study.

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Remarks

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Errata

In the original paper **I**, in Table 3, the percentage for the patients in the OG, concerning two parameters and three or more parameters, should be 31,8 % (14/44) and 29,5 % (13/44), respectively.

In the original paper **III**, last sentence, first page, it says: However, PL is, due to its strong negative…It should be *positive* as said in the rest of the paper.

In Table 4, the number in the column of survival for group 15 should be 6.

In the original paper **V, i**n Table 2, the concentration of NaCl should read 9 mg/ml.

References

1. Markogiannakis H, Messaris E, Dardamanis D, Pararas N, Tzertzemeles D, Giannopoulos P et al. Acute mechanical bowel obstruction: clinical presentation, etiology, management and outcome. World J Gastroenterol 2007;13:432-7.

2. Miller G, Boman J, Shrier I, Gordon PH. Etiology of small bowel obstruction. Am J Surg 2000;180:33-6.

3. Menzies D. Postoperative adhesions: their treatment and relevance in clinical practice. Ann R Coll Surg Engl 1993;75:147-53.

4. Ellis H. The clinical significance of adhesions: focus on intestinal obstruction. Eur J Surg Suppl 1997;577:5-9.

5. Ellis H, Moran BJ, Thompson JN, Parker MC, Wilson MS, Menzies D et al. Adhesion-related hospital readmissions after abdominal and pelvic surgery: a retrospective cohort study. Lancet 1999;353:1476-80.

6. Cox MR, Gunn IF, Eastman MC, Hunt RF, Heinz AW. The operative aetiology and types of adhesions causing small bowel obstruction. ANZ J Surg 1993;63:848-52.

7. Fazio VW, Ziv Y, Church JM, Oakley JR, Lavery IC, Milsom JW and Schroeder TK.. Ileal pouch-anal anastomoses complications and function in 1005 patients. Ann Surg 1995;222:120-7.

8. Fazio VW, Kiran RP, Remzi FH, Coffey JC, Heneghan HM, Kirat HT et al. Ileal pouch anal anastomosis: analysis of outcome and quality of life in 3707 patients. Ann Surg 2013;257:679-85.

9. Hahnloser D, Pemberton JH, Wolff BG, Larson DR, Crownhart BS, Dozois RR. Results at up to 20 years after ileal pouch-anal anastomosis for chronic ulcerative colitis. Br J Surg 2007;94:333-40.

10. Foster NM, McGory ML, Zingmond DS, Ko CY. Small bowel obstruction: a population-based appraisal. J Am Coll Surg 2006;203:170-6.

11. Grassi R, Romano S, D'Amario F, Giorgio Rossi A, Romano L, Pinto F et al. The relevance of free fluid between intestinal loops detected by sonography in the clinical assessment of small bowel obstruction in adults. Eur J Radiol 2004;50:5-14.

12. Obuz F, Terzi C, Sokmen S, Yilmaz E, Yildiz D, Fuzun M. The efficacy of helical CT in the diagnosis of small bowel obstruction. Eur J Radiol 2003;48:299-304.

13. Zalcman M, Sy M, Donckier V, Closset J, Gansbeke DV. Helical CT signs in the diagnosis of intestinal ischemia in small-bowel obstruction. AJR Am J Roentgenol 2000;175:1601-7.

14. Di Saverio S, Coccolini F, Galati M, Smerieri N, Biffl WL, Ansaloni L et al. Bologna guidelines for diagnosis and management of adhesive small bowel obstruction (ASBO): 2013 update of the evidence-based guidelines from the world society of emergency surgery ASBO working group. World J Emerg Surg 2013;8:42.

15. Tresallet C, Lebreton N, Royer B, Leyre P, Godiris-Petit G, Menegaux F. Improving the management of acute adhesive small bowel obstruction with CT-scan and water-soluble contrast medium: a prospective study. Dis Colon Rectum 2009;52:1869-76.

16. Choi HK CK, Law WL. Therapeutic value of gastrografin in adhesive small bowel obstruction after unsuccessful conservative treatment: a prospective randomized trial. Ann Surg 2002;236:1-6.

17. Srinivasa S, Thakore N, Abbas S, Mahmood M, Kahokehr AA, Hill AG. Impact of gastrografin in clinical practice in the management of adhesive small bowel obstruction. Can J Surg 2011;54:123-7.

18. Wadani HA, Al Awad NI, Hassan KA, Zakaria HM, Alaqeel FO. Role of water soluble contrast agents in assigning patients to a non-operative course in adhesive small bowel obstruction. Oman medical journal 2011;26:454-6.

19. Fevang BT, Jensen D, Svanes K, Viste A. Early operation or conservative management of patients with small bowel obstruction? Eur J Surg 2002;168:475-81.

20. Di Saverio S, Catena F, Ansaloni L, Gavioli M, Valentino M, Pinna AD. Water-soluble contrast medium (gastrografin) value in adhesive small intestine obstruction (ASIO): a prospective, randomized, controlled, clinical trial. World J Surg 2008;32:2293-304.

21. Burge J, Abbas SM, Roadley G, Donald J, Connolly A, Bisset IP et al. Randomized controlled trial of Gastrografin in adhesive small bowel obstruction. ANZ J Surg 2005;75:672-4.

22. Abbas S BI, Parry BR. Oral water soluble contrast for the management of adhesive small bowel obstruction. Cochrane Database Syst Rev 2007 update from 2005;18:CD004651.

23. Ambiru S, Furuyama N, Kimura F, Shimizu H, Yoshidome H, Miyazaki M et al. Effect of hyperbaric oxygen therapy on patients with adhesive intestinal obstruction associated with abdominal surgery who have failed to respond to more than 7 days of conservative treatment. Hepato-gastroenterology 2008;55:491-5.

24. Williams SB GJ, Young HA, Orkin BA. Small bowel obstruction: conservative vs. surgical management. Dis Colon Rectum 2005;48:1140-6.

25. Miller G, Boman J, Shrier I, Gordon PH. Natural history of patients with adhesive small bowel obstruction. Br J Surg 2000;87:1240-7.

26. Chen SC, Lin FY, Lee PH, Yu SC, Wang SM, Chang KJ. Water-soluble contrast study predicts the need for early surgery in adhesive small bowel obstruction. Br J Surg 1998;85:1692-4.

27. Beck DE, Ferguson MA, Opelka FG, Fleshman JW, Gervaz P, Wexner SD. Effect of previous surgery on abdominal opening time. Dis Colon Rectum 2000;43:1749-53.

28. Coleman MG, McLain AD, Moran BJ. Impact of previous surgery on time taken for incision and division of adhesions during laparotomy. Dis Colon Rectum 2000;43:1297-9.

29. Van Der Krabben AA, Dijkstra FR, Nieuwenhuijzen M, Reijnen MM, Schaapveld M, Van Goor H. Morbidity and mortality of inadvertent enterotomy during adhesiotomy. Br J Surg 2000;87:467-71.

30. Franko J, O'Connell BG, Mehall JR, Harper SG, Nejman JH, Zebley DM et al. The influence of prior abdominal operations on conversion and complication rates in laparoscopic colorectal surgery. JSLS 2006;10:169-75.

31. van Goor H. Consequences and complications of peritoneal adhesions. Colorectal Dis 2007;9 Suppl 2:25-34.

32. Duron JJ, Silva NJ, du Montcel ST, Berger A, Muscari F, Hennet H et al. Adhesive postoperative small bowel obstruction: incidence and risk factors of recurrence after surgical treatment: a multicenter prospective study. Ann Surg 2006;244:750-7.

33. Schraufnagel D, Rajaee S, Millham FH. How many sunsets? Timing of surgery in adhesive small bowel obstruction: a study of the Nationwide Inpatient Sample. J Trauma Acute Care Surg 2013;74:181-7; discussion 7-9.

34. Fevang BT FJ, Stangeland L, Soreide O, Svanes K, Viste A. Complications and death after surgical treatment of small bowel obstruction: A 35-year institutional experience. Ann Surg 2000;231:529-37.

35. Leung AM, Vu H. Factors predicting need for and delay in surgery in small bowel obstruction. Am Surg 2012;78:403-7.

36. Cosse C, Regimbeau JM, Fuks D, Mauvais F, Scotte M. Serum procalcitonin for predicting the failure of conservative management and the need for bowel resection in patients with small bowel obstruction. J Am Coll Surg 2013;216:997-1004.

37. Chen SC, Lee CC, Hsu CY, Yen ZS, Fang CC, Ma MH et al. Progressive increase of bowel wall thickness is a reliable indicator for surgery in patients with adhesive small bowel obstruction. Dis Colon Rectum 2005;48:1764-71.

38. Lappas JC RB, Maglinte DT. Abdominal Radiography Findings in Small-Bowel Obstruction. Am J Roentgenol 2001;176:167-74.

39. Thompson WM, Kilani RK, Smith BB, et al. Accuracy of abdominal radiography in acute small-bowel obstruction: does reviewer experience matter? AJR American journal of roentgenology 2007;188:W233-8.

40. Addiss DG, Shaffer N, Fowler BS, Tauxe RV. The epidemiology of appendicitis and appendectomy in the United States. Am J Epidemiol 1990;132:910-25.

41. Andersson R, Hugander A, Thulin A, Nystrom PO, Olaison G. Indications for operation in suspected appendicitis and incidence of perforation. BMJ 1994;308:107-10.

42. Stein GY, Rath-Wolfson L, Zeidman A, Atar E, Marcus O, Joubran S et al. Sex differences in the epidemiology, seasonal variation, and trends in the management of patients with acute appendicitis. Langenbeck's Arch Surg 2012;397:1087-92.

43. Andersson RE. The natural history and traditional management of appendicitis revisited: spontaneous resolution and predominance of prehospital perforations imply that a correct diagnosis is more important than an early diagnosis. World J Surg 2007;31:86-92.

44. Tingstedt B, Johansson J, Nehez L, Andersson R. Late abdominal complaints after appendectomy--readmissions during long-term follow-up. Dig Sur 2004;21:23-7.

45. Andersson RE. Small bowel obstruction after appendicectomy. Br J Surg 2001;88:1387-91.

46. Blomqvist PG, Andersson RE, Granath F, Lambe MP, Ekbom AR. Mortality after appendectomy in Sweden, 1987-1996. Ann Surg 2001;233:455-60.

47. Leung TT, Dixon E, Gill M, Mador BD, Moulton KM, Kaplan GG et al. Bowel obstruction following appendectomy: what is the true incidence? Ann Surg 2009;250:51-3.

48. Tingstedt B, Andersson R. Improved diagnostic accuracy in patients with suspected appendicitis Ann Gastroenterol 2005;18:65-9.

49. Moberg AC, Berndsen F, Palmquist I, Petersson U, Resch T, Montgomery A. Randomized clinical trial of laparoscopic versus open appendicectomy for confirmed appendicitis. Br J Surg 2005;92:298-304.

50. Busch M, Gutzwiller FS, Aellig S, Kuettel R, Metzger U, Zingg U. In-hospital delay increases the risk of perforation in adults with appendicitis. World J Surg 2011;35:1626-33.

51. Flum DR, Morris A, Koepsell T, Dellinger EP. Has misdiagnosis of appendicitis decreased over time? A population-based analysis. JAMA 2001;286:1748-53.

52. Pittman-Waller VA, Myers JG, Stewart RM, Dent DL, Page CP, Gray GA et al. Appendicitis: why so complicated? Analysis of 5755 consecutive appendectomies. Am Surg 2000;66:548-54.

53. Alvarado A. A practical score for the early diagnosis of acute appendicitis. Ann Emerg Med 1986;15:557-64.

54. Andersson M, Andersson RE. The appendicitis inflammatory response score: a tool for the diagnosis of acute appendicitis that outperforms the Alvarado score. World J Surg 2008;32:1843-9.

55. de Castro SM, Unlu C, Steller EP, van Wagensveld BA, Vrouenraets BC. Evaluation of the appendicitis inflammatory response score for patients with acute appendicitis. World J Surg 2012;36:1540-5.

56. Andersson RE. Evaluation of the appendicitis inflammatory response score for patients with acute appendicitis. World J Surg 2012;36:1546-7.

57. Orr RK, Porter D, Hartman D. Ultrasonography to evaluate adults for appendicitis: decision making based on meta-analysis and probabilistic reasoning. Acad Emerg Med 1995;2:644-50.

58. Rao PM, Rhea JT, Rattner DW, Venus LG, Novelline RA. Introduction of appendiceal CT: impact on negative appendectomy and appendiceal perforation rates. Ann Surg 1999;229:344-9.

59. Toorenvliet BR, Wiersma F, Bakker RF, Merkus JW, Breslau PJ, Hamming JF. Routine ultrasound and limited computed tomography for the diagnosis of acute appendicitis. World J Surg 2010;34:2278-85.

60. Terasawa T, Blackmore CC, Bent S, Kohlwes RJ. Systematic review: computed tomography and ultrasonography to detect acute appendicitis in adults and adolescents. Ann Intern Med 2004;141:537-46.

61. Styrud J, Eriksson S, Nilsson I, Ahlberg G, Haapaniemi S, Neovius G et al. Appendectomy versus antibiotic treatment in acute appendicitis. a prospective multicenter randomized controlled trial. World J Surg 2006;30:1033-7.

62. Hansson J, Korner U, Ludwigs K, Johnsson E, Jonsson C, Lundholm K. Antibiotics as first-line therapy for acute appendicitis: evidence for a change in clinical practice. World J Surg 2012;36:2028-36.

63. Hansson J, Korner U, Khorram-Manesh A, Solberg A, Lundholm K. Randomized clinical trial of antibiotic therapy versus appendicectomy as primary treatment of acute appendicitis in unselected patients. Br J Surg 2009;96:473-81.

64. Ansaloni L, Catena F, Coccolini F, Ercolani G, Gazzotti F, Pasqualini E et al. Surgery versus conservative antibiotic treatment in acute appendicitis: a systematic review and meta-analysis of randomized controlled trials. Dig Surg 2011;28:210-21.

65. www.socialstyrelsen.se/statistic/statistikdatabas.Access date Dec 15, 2013.

66. Bisset AF. Appendicectomy in Scotland: a 20-year epidemiological comparison. J Publ Health Med 1997;19:213-8.

67. Faiz O, Clark J, Brown T, Bottle A, Antoniou A, Farrands P et al. Traditional and laparoscopic appendectomy in adults: outcomes in English NHS hospitals between 1996 and 2006. Ann Surg 2008;248:800-6.

68. Flum DR, Koepsell T. The clinical and economic correlates of misdiagnosed appendicitis: nationwide analysis. Arch Surg 2002;137:799-804; discussion

69. Brockman SF, Scott S, Guest GD, Stupart DA, Ryan S, Watters DA. Does an Acute Surgical Model increase the rate of negative appendicectomy or perforated appendicitis? ANZ J Surg 2013;83:744-7.

70. Jones PF. Suspected acute appendicitis: trends in management over 30 years. Br J Surg 2001;88:1570-7.

71. Graff L, Russell J, Seashore J, Tate J, Elwell A, Prete M et al. False-negative and false-positive errors in abdominal pain evaluation: failure to diagnose acute appendicitis and unnecessary surgery. Acad Emerg Med 2000;7:1244-55.

72. Rogozov V, Bermel N. Auto-appendectomy in the Antarctic: case report. BMJ 2009;339:b4965.

73. McBurney C. IV. The Incision Made in the Abdominal Wall in Cases of Appendicitis, with a Description of a New Method of Operating. Ann Surg 1894;20:38-43.

74. Semm K. Endoscopic appendectomy. Endoscopy 1983;15:59-64.

75. Sauerland S, Jaschinski T, Neugebauer EA. Laparoscopic versus open surgery for suspected appendicitis. Cochrane Database Syst Rev 2010:CD001546.

76. Guller U, Hervey S, Purves H, Muhlbaier LH, Peterson ED, Eubanks S et al. Laparoscopic versus open appendectomy: outcomes comparison based on a large administrative database. Ann Surg 2004;239:43-52.

77. Golub R, Siddiqui F, Pohl D. Laparoscopic versus open appendectomy: a metaanalysis. J Am Coll Surg 1998;186:545-53.

78. Wei B, Qi CL, Chen TF, Zheng ZH, Huang JL, Hu BG et al. Laparoscopic versus open appendectomy for acute appendicitis: a metaanalysis. Surg Endosc 2011;25:1199-208.

79. Moberg AC, Ahlberg G, Leijonmarck CE, Montgomery A, Reiertsen O, Rosseland AR et al. Diagnostic laparoscopy in 1043 patients with suspected acute appendicitis. Eur J Surg 1998;164:833-40; discussion 41.

80. Teh SH, O'Ceallaigh S, McKeon JG, O'Donohoe MK, Tanner WA, Keane FB. Should an appendix that looks 'normal' be removed at diagnostic laparoscopy for acute right iliac fossa pain? Eur J Surg 2000;166:388-9.

81. Thorell A, Grondal S, Schedvins K, Wallin G. Value of diagnostic laparoscopy in fertile women with suspected appendicitis. Eur J Surg 1999;165:751-4.

82. Kraemer M, Ohmann C, Leppert R, Yang Q. Macroscopic assessment of the appendix at diagnostic laparoscopy is reliable. Surg Endosc 2000;14:625-33.

83. van den Broek WT, Bijnen AB, de Ruiter P, Gouma DJ. A normal appendix found during diagnostic laparoscopy should not be removed. Br J Surg 2001;88:251-4.

84. Masoomi H, Mills S, Dolich MO, Ketana N, Carmichael JC, Nguyen NT et al. Comparison of outcomes of laparoscopic versus open appendectomy in adults: data from the Nationwide Inpatient Sample (NIS), 2006-2008. J Gastrointestl Surg 2011;15:2226-31.

85. Tiwari MM, Reynoso JF, Tsang AW, Oleynikov D. Comparison of outcomes of laparoscopic and open appendectomy in management of uncomplicated and complicated appendicitis. Ann Surg 2011;254:927-32.

86. Andersson RE. Short and long-term mortality after appendectomy in Sweden 1987 to 2006. Influence of appendectomy diagnosis, sex, age, co-morbidity, surgical method, hospital volume, and time period. A national population-based cohort study. World J Surg 2013;37:974-81.

87. Ahlberg G, Bergdahl S, Rutqvist J, Soderquist C, Frenckner B. Mechanical small-bowel obstruction after conventional appendectomy in children. Eur J Pediatr Surg 1997;7:13-5.

88. Riber C, Soe K, Jorgensen T, Tonnesen H. Intestinal obstruction after appendectomy. Scand J Gastroenterol 1997;32:1125-8.

89. Zbar RI, Crede WB, McKhann CF, Jekel JF. The postoperative incidence of small bowel obstruction following standard, open appendectomy and cholecystectomy: a six-year retrospective cohort study at Yale-New Haven Hospital. Conn Med 1993;57:123-7.

90. Sykes PA, Schofield PF. Early postoperative small bowel obstruction. Br J Surg 1974;61:594-600.

91. Stewart RM, Page CP, Brender J, Schwesinger W, Eisenhut D. The incidence and risk of early postoperative small bowel obstruction. A cohort study. Am J Surg 1987;154:643-7.

92. Tsao KJ, St Peter SD, Valusek PA, Keckler SJ, Sharp S, Holcomb GW et al. Adhesive small bowel obstruction after appendectomy in children: comparison between the laparoscopic and open approach. J Pediatr Surg 2007;42:939-42; discussion 42.

93. Kaselas C, Molinaro F, Lacreuse I, Becmeur F. Postoperative bowel obstruction after laparoscopic and open appendectomy in children: a 15-year experience. J Pediatr Surg 2009;44:1581-5.

94. Markar SR, Blackburn S, Cobb R, Karthikesalingam A, Evans J, Kinross J et al. Laparoscopic versus open appendectomy for complicated and uncomplicated appendicitis in children. J Gastrointest Surg 2012;16:1993-2004.

95. Angenete E, Jacobsson A, Gellerstedt M, Haglind E. Effect of laparoscopy on the risk of small-bowel obstruction: a population-based register study. Arch Surg 2012;147:359-65.

96. Swank HA, Eshuis EJ, van Berge Henegouwen MI, Bemelman WA. Short- and long-term results of open versus laparoscopic appendectomy. World J Surg 2011;35:1221-6; discussion 7-8.

97. Kouhia ST, Heiskanen JT, Huttunen R, Ahtola HI, Kiviniemi VV, Hakala T. Long-term follow-up of a randomized clinical trial of open versus laparoscopic appendicectomy. Br J Surg 2010;97:1395-400.

98. Barmparas G, Branco BC, Schnuriger B, Lam L, Inaba K, Demetriades D. The incidence and risk factors of post-laparotomy adhesive small bowel obstruction. J Gastrointest Surg 2010;14:1619-28.

99. Pawlaczyk K, Kuzlan M, Wieczorowska-Tobis K, Pawlik-Juzkow H, Breborovicz A, Knapowski J et al. Species-dependent topography of the peritoneum. Adv Perit Dial 1996;12:3-6.

100. Hall JC, Heel KA, Papadimitriou JM, Platell C. The pathobiology of peritonitis. Gastroenterology 1998;114:185-96.

101. Whitaker D, Papadimitriou JM, Walters MN. The mesothelium and its reactions: a review. Crit Rev Toxicol 1982;10:81-144.

102. Beavis J, Harwood JL, Coles GA, Williams JD. Synthesis of phospholipids by human peritoneal mesothelial cells. Perit Dial Int 1994;14:348-55.

103. Nakatani T, Ohtani O, Tanaka S. Lymphatic stomata in the murine diaphragmatic peritoneum: the timing of their appearance and a map of their distribution. Anat Rec 1996;244:529-39.

104. Di Paolo N, Buoncristiani U, Capotondo L, Gaggiotti E, De Mia M, Rossi P et al. Phosphatidylcholine and peritoneal transport during peritoneal dialysis. Nephron 1986;44:365-70.

105. Bouckaert PX, Evers JL, Doesburg WH, Schellekens LA, Brombacher PH, Rolland R. Patterns of changes in proteins in the peritoneal fluid of women during the periovulatory phase of the menstrual cycle. J Reprod Fertil 1986;77:329-36.

106. Holmdahl L, Ivarsson ML. The role of cytokines, coagulation, and fibrinolysis in peritoneal tissue repair. Eur J Surg 1999;165:1012-9.

107. Hellebrekers BW, Kooistra T. Pathogenesis of postoperative adhesion formation. Br J Surg 2011;98:1503-16.

108. diZerega GS. Biochemical events in peritoneal tissue repair. Eur J Surg Suppl 1997:10-6.

109. Holmdahl L, Eriksson E, Eriksson BI, Risberg B. Depression of peritoneal fibrinolysis during operation is a local response to trauma. Surgery 1998;123:539-44.

110. Scott-Coombes DM, Whawell SA, Thompson JN. The operative peritoneal fibrinolytic response to abdominal operation. Eur J Surg 1995;161:395-9.

111. Holmdahl L, Eriksson E, al-Jabreen M, Risberg B. Fibrinolysis in human peritoneum during operation. Surgery 1996;119:701-5.

112. Ivarsson ML, Bergstrom M, Eriksson E, Risberg B, Holmdahl L. Tissue markers as predictors of postoperative adhesions. Br J Surg 1998;85:1549-54.

113. Holmdahl L. The role of fibrinolysis in adhesion formation. Eur J Surg Suppl 1997:24-31.

114. Brokelman WJ, Holmdahl L, Bergstrom M, Falk P, Klinkenbijl JH, Reijnen MM. Peritoneal fibrinolytic response to various aspects of laparoscopic surgery: a randomized trial. J Surg Res 2006;136:309-13.

115. Brokelman WJ, Holmdahl L, Janssen IM, Falk P, Bergström M, Klinkenbijl JH et al. Decreased peritoneal tissue plasminogen activator during prolonged laparoscopic surgery. J Surg Res 2009;151:89-93.

116. Menzies D. Adhesions: the cellular science. Hosp Med 2004;65:337-9.

117. diZerega GS, Campeau JD. Peritoneal repair and post-surgical adhesion formation. Hum Reprod Update 2001;7:547-55.

118. Ryan GB, Grobety J, Majno G. Postoperative peritoneal adhesions. A study of the mechanisms. Am J Pathol 1971;65:117-48.

119. Nisell H, Larsson B. Role of blood and fibrinogen in development of intraperitoneal adhesions in rats. Fertil Steril 1978;30:470-3.

120. Tingstedt B, Isaksson J, Andersson R. Long-term follow-up and cost analysis following surgery for small bowel obstruction caused by intra-abdominal adhesions. Br J Surg 2007;94:743-8.

121. Weibel MA, Majno G. Peritoneal adhesions and their relation to abdominal surgery. A postmortem study. Am J Surg 1973;126:345-53.

122. Menzies D, Ellis H. Intestinal obstruction from adhesions--how big is the problem? Ann R Coll Rurg Engl 1990;72:60-3.

123. Fevang BT, Fevang J, Lie SA, Soreide O, Svanes K, Viste A. Long-term prognosis after operation for adhesive small bowel obstruction. Ann Surg 2004;240:193-201.

124. Grant HW, Parker MC, Wilson MS, Menzies D, Sunderland G, Thompson JN et al. Population-based analysis of the risk of adhesion-related readmissions after abdominal surgery in children. J Pediatr Surg 2006;41:1453-6.

125. Milingos S, Kallipolitis G, Loutradis D, Liapi A, Mavrommatis K, Drakakis P et al. Adhesions: laparoscopic surgery versus laparotomy. Ann N Y Acad Sci 2000;900:272-85.

126. Monk BJ, Berman ML, Montz FJ. Adhesions after extensive gynecologic surgery: clinical significance, etiology, and prevention. Am J Obstet Gynecol 1994;170:1396-403.

127. Singhal V, Li TC, Cooke ID. An analysis of factors influencing the outcome of 232 consecutive tubal microsurgery cases. Br J Obstet Gynecol 1991;98:628-36.

128. Diamond MP, Freeman ML. Clinical implications of postsurgical adhesions. Hum Reprod Update 2001;7:567-76.

129. Nagata Y, Honjou K, Sonoda M, Makino I, Tamura R, Kawarabayashi T. Peri-ovarian adhesions interfere with the diffusion of gonadotrophin into the follicular fluid. Hum Reprod 1998;13:2072-6.

130. Bouyer J, Coste J, Shojaei T, Pouly JL, Fernandez H, Gerbaud L et al. Risk factors for ectopic pregnancy: a comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol 2003;157:185-94.

131. Oelsner G, Sivan E, Goldenberg M, Carp H, Admon D, Mashiach S. Should lysis of adhesions be performed when in-vitro fertilization and embryo transfer are available? Hum Reprod 1994;9:2339-41.

132. Sulaiman H, Gabella G, Davis MC, Mutsaers SE, Boulos P, Laurent GJ et al. Presence and distribution of sensory nerve fibers in human peritoneal adhesions. Ann Surg 2001;234:256-61.

133. Duffy DM, diZerega GS. Adhesion controversies: pelvic pain as a cause of adhesions, crystalloids in preventing them. J Reprod Med 1996;41:19-26.

134. Paajanen H, Julkunen K, Waris H. Laparoscopy in chronic abdominal pain: a prospective nonrandomized long-term follow-up study. J Clin Gastroenterol 2005;39:110-4.

135. Vrijland WW, Jeekel J, van Geldorp HJ, Swank DJ, Bonjer HJ. Abdominal adhesions: intestinal obstruction, pain, and infertility. Surg Endosc 2003;17:1017-22.

136. Alexander-Williams J. Do adhesions cause pain? Br Med J (Clin Res Ed) 1987;294:659-60.

137. Swank DJ, Swank-Bordewijk SC, Hop WC, van Erp WF, Janssen IM, Bonjer HJ et al. Laparoscopic adhesiolysis in patients with chronic abdominal pain: a blinded randomised controlled multi-centre trial. Lancet 2003;361:1247-51.

138. Sandler RS, Stewart WF, Liberman JN, Ricci JA, Zorich NL. Abdominal pain, bloating, and diarrhea in the United States: prevalence and impact. Dig Dis Sci 2000;45:1166-71.

139. Ray NF, Denton WG, Thamer M, Henderson SC, Perry S. Abdominal adhesiolysis: inpatient care and expenditures in the United States in 1994. J Am Coll Surg 1998;186:1-9.

140. Ivarsson ML, Holmdahl L, Franzen G, Risberg B. Cost of bowel obstruction resulting from adhesions. Eur J Surg 1997;163:679-84.

141. Kossi J, Salminen P, Rantala A, Laato M. Population-based study of the surgical workload and economic impact of bowel obstruction caused by postoperative adhesions. Br J Surg 2003;90:1441-4.

142. Sikirica V, Bapat B, Candrilli SD, Davis KL, Wilson M, Johns A. The inpatient burden of abdominal and gynecological adhesiolysis in the US. BMC surgery 2011;11:13.

143. Schreinemacher MH, ten Broek RP, Bakkum EA, van Goor H, Bouvy ND. Adhesion awareness: a national survey of surgeons. World J Surg 2010;34:2805-12.

144. Meuleman T, Schreinemacher MH, van Goor H, Bakkum EA, Dorr PJ. Adhesion awareness: a nationwide survey of gynaecologists. Eur J Obstet Gynecol Reprod Biol 2013;169:353-9.

145. Holmadhl L, al-Jabreen M, Xia G, Risberg B. The impact of starch-powdered gloves on the formation of adhesions in rats. Eur J Surg 1994;160:257-61.

146. van den Tol MP, Haverlag R, van Rossen ME, Bonthuis F, Marquet RL, Jeekel J. Glove powder promotes adhesion formation and facilitates tumour cell adhesion and growth. Br J Surg 2001;88:1258-63.

147. Risberg B. Adhesions: preventive strategies. Eur J Surg Suppl 1997:32-9.

148. Cooke SA, Hamilton DG. The significance of starch powder contamination in the aetiology of peritoneal adhesions. Br J Surg 1977;64:410-2.

149. Komoto Y, Shimoya K, Shimizu T, Kimura T, Hayashi S, Temma-Asano K et al. Prospective study of non-closure or closure of the peritoneum at cesarean delivery in 124 women: Impact of prior peritoneal closure at primary cesarean on the interval time between first cesarean section and the next pregnancy and significant adhesion at second cesarean. J Obstet Gynecol Res 2006;32:396-402.

150. Malvasi A, Tinelli A, Farine D, Rahimi S, Cavallotti C, Vergara D et al. Effects of visceral peritoneal closure on scar formation at cesarean delivery. Int J Gynecol Obstet 2009;105:131-5.

151. Gutt CN, Oniu T, Schemmer P, Mehrabi A, Buchler MW. Fewer adhesions induced by laparoscopic surgery? Surg Endosc 2004;18:898-906.

152. Lundorff P, Hahlin M, Kallfelt B, Thorburn J, Lindblom B. Adhesion formation after laparoscopic surgery in tubal pregnancy: a randomized trial versus laparotomy. Fertil Steril 1991;55:911-5.

153. Luijendijk RW, de Lange DC, Wauters CC, Hop WC, Duron JJ, Pailler JL et al. Foreign material in postoperative adhesions. Ann Surg 1996;223:242-8.

154. Kavic SM. Adhesions and adhesiolysis: the role of laparoscopy. JSLS 2002;6:99-109.

155. Bergstrom M, Ivarsson ML, Holmdahl L. Peritoneal response to pneumoperitoneum and laparoscopic surgery. Br J Surg 2002;89:1465-9.

156. Bergstrom M, Falk P, Holmdahl L. CO2 promotes plasminogen activator inhibitor type 1 expression in human mesothelial cells. Surg Endosc 2003;17:1818-22.

157. Gray RI, Ott DE, Henderson AC, Cochran SA, Roth EA. Severe local hypothermia from laparoscopic gas evaporative jet cooling: a mechanism to explain clinical observations. JSLS 1999;3:171-7.

158. Tingstedt B, Isaksson K, Andersson E, Andersson R. Prevention of abdominal adhesions--present state and what's beyond the horizon? Eur Surg Res 2007;39:259-68.

159. Tang CL, Jayne DG, Seow-Choen F, Ng YY, Eu KW, Mustapha N. A randomized controlled trial of 0.5% ferric hyaluronate gel (Intergel) in the prevention of adhesions following abdominal surgery. Ann Surg 2006;243:449-55.

160. Wilson MS, Menzies D, Knight AD, Crowe AM. Demonstrating the clinical and cost effectiveness of adhesion reduction strategies. Colorectal Dis 2002;4:355-60.

161. ten Broek RP, Stommel MW, Strik C, van Laarhoven CJ, Keus F, van Goor H. Benefits and harms of adhesion barriers for abdominal surgery: a systematic review and meta-analysis. Lancet 2014;383:48-59.

162. Kumar S, Wong PF, Leaper DJ. Intra-peritoneal prophylactic agents for preventing adhesions and adhesive intestinal obstruction after non-gynaecological abdominal surgery. Cochrane Database Syst Rev 2009:CD005080.

163. Fazio VW, Cohen Z, Fleshman JW, van Goor H, Bauer JJ, Wolff BG et al. Reduction in adhesive small-bowel obstruction by Seprafilm adhesion barrier after intestinal resection. Dis Colon Rectum 2006;49:1-11.

164. Beck DE, Cohen Z, Fleshman JW, Kaufman HS, van Goor H, Wolff BG. A prospective, randomized, multicenter, controlled study of the safety of Seprafilm adhesion barrier in abdominopelvic surgery of the intestine. Dis Colon Rectum 2003;46:1310-9.

165. Vrijland WW, Tseng LN, Eijkman HJ, Jakimowicz JJ, Lequit P, Stassen LP et al. Fewer intraperitoneal adhesions with use of hyaluronic acid-carboxymethylcellulose membrane: a randomized clinical trial. Ann Surg 2002;235:193-9.

166. Prevention of postsurgical adhesions by INTERCEED(TC7), an absorbable adhesion barrier: a prospective randomized multicenter clinical study. INTERCEED(TC7) Adhesion Barrier Study Group. Fertil Steril 1989;51:933-8.

167. Azziz R. Microsurgery alone or with INTERCEED Absorbable Adhesion Barrier for pelvic sidewall adhesion re-formation. The INTERCEED (TC7) Adhesion Barrier Study Group II. Surg Gynecol Obstet 1993;177:135-9.

168. Wiseman DM, Trout JR, Franklin RR, Diamond MP. Metaanalysis of the safety and efficacy of an adhesion barrier (Interceed TC7) in laparotomy. J Reprod Med 1999;44:325-31.

169. Sawada T, Nishizawa H, Nishio E, Kadowaki M. Postoperative adhesion prevention with an oxidized regenerated cellulose adhesion barrier in infertile women. The J Reprod Med 2000;45:387-9.

170. Wiseman DM, Gottlick-Iarkowski L, Kamp L. Effect of different barriers of oxidized regenerated cellulose (ORC) on cecal and sidewall adhesions in the presence and absence of bleeding. J Invest Surg 1999;12:141-6.

171. Muller SA, Treutner KH, Haase G, Kinzel S, Tietze L, Schumpelick V. Effect of intraperitoneal antiadhesive fluids in a rat peritonitis model. Arch Surg 2003;138:286-90.

172. Rodgers KE, Verco SJ, diZerega GS. Effects of intraperitoneal 4% icodextrin solution on the healing of bowel anastomoses and laparotomy incisions in rabbits. Colorectal Dis 2003;5:324-30.

173. van den Tol P, ten Raa S, van Grevenstein H, Marquet R, van Eijck C, Jeekel H. Icodextrin reduces postoperative adhesion formation in rats without affecting peritoneal metastasis. Surgery 2005;137:348-54.

174. Brown CB, Luciano AA, Martin D, Peers E, Scrimgeour A, diZerega GS. Adept (icodextrin 4% solution) reduces adhesions after laparoscopic surgery for adhesiolysis: a double-blind, randomized, controlled study. Fertil Steril 2007;88:1413-26.

175. diZerega GS, Verco SJ, Young P, Kettle M, Kobak W, Martin D et al. A randomized, controlled pilot study of the safety and efficacy of 4% icodextrin solution in the reduction of adhesions following laparoscopic gynaecological surgery. Hum Reprod 2002;17:1031-8.

176. Menzies D, Pascual MH, Walz MK, Duron JJ, Tonelli F, Crowe A et al. Use of icodextrin 4% solution in the prevention of adhesion formation following general surgery: from the multicentre ARIEL Registry. Ann R Coll Surg Engl 2006;88:375-82.

177. Catena F, Ansaloni L, Di Saverio S, Pinna AD. P.O.P.A. study: prevention of postoperative abdominal adhesions by icodextrin 4% solution after laparotomy for adhesive small bowel obstruction. A prospective randomized controlled trial. J Gastrointest Surg 2012;16:382-8.

178. Tjandra JJ, Chan MK. A sprayable hydrogel adhesion barrier facilitates closure of defunctioning loop ileostomy: a randomized trial. Dis Colon Rectum 2008;51:956-60.

179. Mettler L, Hucke J, Bojahr B, Tinneberg HR, Leyland N, Avelar R. A safety and efficacy study of a resorbable hydrogel for reduction of post-operative adhesions following myomectomy. Hum Reprod 2008;23:1093-100.

180. King A, Strand B, Rokstad AM, Kulseng B, Andersson A, Skjåk-Braek G et al. Improvement of the biocompatibility of alginate/poly-L-lysine/alginate microcapsules by the use of epimerized alginate as a coating. J Biomed Mater Res A 2003;64:533-9.

181. Takei Y, Maruyama A, Ferdous A, Nishimura Y, Kawano S, Ikejima K et al. Targeted gene delivery to sinusoidal endothelial cells: DNA nanoassociate bearing hyaluronan-glycocalyx. FASEB J 2004;18:699-701.

182. Geornaras I, Yoon Y, Belk KE, Smith GC, Sofos JN. Antimicrobial activity of epsilon-polylysine against Escherichia coli O157:H7, Salmonella Typhimurium, and Listeria monocytogenes in various food extracts. J Food Sci 2007;72:M330-4.

183. Billinger M, Buddeberg F, Hubbell JA, Elbert DL, Schaffner T, Mettler D et al. Polymer stent coating for prevention of neointimal hyperplasia. J Invasive Cardiol 2006;18:423-6; discussion 7.

184. Li C, Yu DF, Newman RA, Cabral F, Stephens LC, Hunter N et al. Complete regression of well-established tumors using a novel water-soluble poly(L-glutamic acid)-paclitaxel conjugate. Cancer Res 1998;58:2404-9.

185. Moroson H. Polycation- treated tumor cells in vivo and in vitro. Cancer research 1971;31:373-80.

186. Nehez L, Vodros D, Axelsson J, Tingstedt B, Lindman B, Andersson R. Prevention of postoperative peritoneal adhesions: effects of lysozyme, polylysine and polyglutamate versus hyaluronic acid. Scand J Gastroenterol 2005;40:1118-23.

187. Nehez L, Tingstedt B, Vodros D, Axelsson J, Lindman B, Andersson R. Novel treatment in peritoneal adhesion prevention: protection by polypeptides. Scand J Gastroenterol 2006;41:1110-7.

188. Larsson K. Lipids-molecular organization, physical functions and technical applications. Dundee, Scotland: The Oily Press 1994:100-6.

189. Menger FM, Seredyuk VA, Kitaeva MV, Yaroslavov AA, Melik-Nubarov NS. Migration of poly-L-lysine through a lipid bilayer. J Am Chem Soc 2003;125:2846-7.

190. Holmdahl L. Making and covering of surgical footprints. Lancet 1999;353:1456-7.

191. Haney AF. Identification of macrophages at the site of peritoneal injury: evidence supporting a direct role for peritoneal macrophages in healing injured peritoneum. Fertil Steril 2000;73:988-95.

192. Mutsaers SE, Whitaker D, Papadimitriou JM. Stimulation of mesothelial cell proliferation by exudate macrophages enhances serosal wound healing in a murine model. Am J Pathol 2002;160:681-92.

193. Akerberg D, Isaksson K, Posaric-Bauden M, Andersson R, Tingstedt B. Effects of Polylysine and Polyglutamate on Inflammation and the Normal Process of Peritoneal Healing After Surgery. J Tissue Sci Eng 2012;3:117.

194. Akerberg D, Grunditz C, Posaric-Bauden M, Isaksson K, Andersson R, Tingstedt B. The influence on abdominal adhesions and inflammation in rabbits after exposure to differently charged polypeptides. JBiSE 2012;5:432-8.

195. Tingstedt B, Nehez L, Axelsson J, Lindman B, Andersson R. Increasing anastomosis safety and preventing abdominal adhesion formation by the use of polypeptides in the rat. Int J Colorect Dis 2006;21:566-72.

196. Tingstedt B, Nehez L, Lindman B, Andersson R. Effect of bioactive polypeptides on leaking large bowel anastomosis and intestines in the rat. Journal of investigative surgery : J Invest Surg 2007;20:229-35.

197. Tingstedt B, Nehez L, Lindman B, Andersson R. Efficacy of bioactive polypeptides on bleeding and intra-abdominal adhesions. Eur Surg Res 2007;39:35-40.

198. Akerberg D, Posaric-Bauden M, Isaksson K, Andersson R, Tingstedt B. Prevention of pleural adhesions by bioactive polypeptides - a pilot study. Int J Med Sci 2013;10:1720-6.

199. Hunter AC. Molecular hurdles in polyfectin design and mechanistic background to polycation induced cytotoxicity. Adv Drug Deliv Rev 2006;58:1523-31.

200. Strand BL, Ryan TL, In't Veld P, Kulseng B, Rokstad AM, Skjak-Braek G et al. Poly-L-Lysine induces fibrosis on alginate microcapsules via the induction of cytokines. Cell Transplant 2001;10:263-75.

201. Jorquera RA, Berrios J, Sans J, Vergara C, Benos DJ, Reyes JG. Permeability changes induced by polylysines in rat spermatids. Biol Cell 2002;94:233-41.

202. Salvi M, Toninello A. Effects of polyamines on mitochondrial Ca(2+) transport. Biochimica et biophysica acta 2004;1661:113-24.

203. Moreau E, Domurado M, Chapon P, Vert M, Domurad D. Biocompatibility of polycations: in vitro agglutination and lysis of red blood cells and in vivo toxicity. J Drug Target 2002;10:161-73.

204. Moreau E, Ferrari I, Drochon A, Chapon P, Vert M, Domurado D. Interactions between red blood cells and a lethal, partly quaternized tertiary polyamine. J Control Release 2000;64:115-28.

205. Kurosaki T, Kitahara T, Kawakami S, Higuchi Y, Yamaguchi A, Nakagawa H et al. Gamma-polyglutamic acid-coated vectors for effective and safe gene therapy. J Control Release 2010;142:404-10.

206. Holmdahl L, al-Jabreen M, Risberg B. Experimental models for quantitative studies on adhesion formation in rats and rabbits. Eur Surg Res 1994;26:248-56.

207. Aldemir M, Yagnur Y, Tacyildir I. The predictive factors for the necessity of operative treatment in adhesive small bowel obstruction cases. Acta Chir Belg 2004;104:76-80.

208. Jancelewicz T, Vu LT, Shawo AE, Yeh B, Gasper WJ, Harris HW. Predicting strangulated small bowel obstruction: an old problem revisited. J Gastrointest Surg 2009;13:93-9.

209. Ten Broek RP, Issa Y, van Santbrink EJ, Bouvy ND, Kruitwagen RF, Jeekel J et al. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. BMJ 2013;347:f5588.

210. Bizer LS, Liebling RW, Delany HM, Gliedman ML. Small bowel obstruction: the role of nonoperative treatment in simple intestinal obstruction and predictive criteria for strangulation obstruction. Surgery 1981;89:407-13.

211. Delabrousse E, Lubrano J, Jehl J, Morati P, Rouget C, Mantion GA et al. Small-bowel obstruction from adhesive bands and matted adhesions: CT differentiation. Am J Roentgenol 2009;192:693-7.

212. Parker MC, Wilson MS, Menzies D, Sunderland G, Clark DN, Knight AD et al. The SCAR-3 study: 5-year adhesion-related readmission risk following lower abdominal surgical procedures. Colorectal Dis 2005;7:551-8.

213. Schnuriger B, Barmparas G, Branco BC, Lustenberger T, Inaba K, Demetriades D. Prevention of postoperative peritoneal adhesions: a review of the literature. Am J Surg 2011;201:111-21.

214. O'Connor DB, Winter DC. Bowel obstruction following appendectomy: a protective role implied for laparoscopy? Ann Surg 2010;251:1190-1; author reply 1.

215. Gupta N, Machado-Aranda D, Bennett K, Mittal VK. Identification of preoperative risk factors associated with the conversion of laparoscopic to open appendectomies. International surgery 2013;98:334-9.

216. Abe T, Nagaie T, Miyazaki M, Ochi M, Fukuya T, Kajiyama K. Risk factors of converting to laparotomy in laparoscopic appendectomy for acute appendicitis. Clin Exp Gastroenterol 2013;6:109-14.

217. Hellberg A, Rudberg C, Enochsson L, Gudbjartson T, Wenner J, Kullman E et al. Conversion from laparoscopic to open appendicectomy: a possible drawback of the laparoscopic technique? Eur J Surg 2001;167:209-13.

218. Ditzel M, van Ginhoven TM, van der Wal JB, Hop W, Coene PP, Lange JF et al. What patients and surgeons should know about the consequences of appendectomy for acute appendicitis after long-term follow-up: factors influencing the incidence of chronic abdominal complaints. J Gastroint Surg 2013;17:1471-6.

219. Vivanti A, Harvey K, Ash S, Battistutta D. Clinical assessment of dehydration in older people admitted to hospital: what are the strongest indicators? Arch Gerontol Geriatr 2008;47:340-55.

220. Sajja SB, Schein M. Early postoperative small bowel obstruction. Br J Surg 2004;91:683-91.

221. Wilson MS, Hawkswell J, McCloy RF. Natural history of adhesional small bowel obstruction: counting the cost. Br J Surg 1998;85:1294-8.

222. Shih IL, Van YT, Shen MH. Biomedical applications of chemically and microbiologically synthesized poly(glutamic acid) and poly(lysine). Mini Rev Med Chem 2004;4:179-88.

223. Shih IL, Shen MH, Van YT. Microbial synthesis of poly(ε-lysine) and its various applications Bioresource Technology 2004;97:1148-59.

224. Shima S, Matsuoka H, Iwamoto T, Sakai H. Antimicrobial action of epsilon-poly-L-lysine. J Antibiot 1984;37:1449-55.

225. Fuchs SM, Raines RT. Pathway for polyarginine entry into mammalian cells. Biochemistry 2004;43:2438-44.

226. Steijns JM, van Hooijdonk AC. Occurrence, structure, biochemical properties and technological characteristics of lactoferrin. Br J Nutr 2000;84 Suppl 1:S11-7.

227. Morgan DM, Larvin VL, Pearson JD. Biochemical characterisation of polycation-induced cytotoxicity to human vascular endothelial cells. J Cell Sci 1989;94 (Pt 3):553-9.

228. Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. Biomaterials 2003;24:1121-31.

229. Arnold LJ, Jr., Dagan A, Gutheil J, Kaplan NO. Antineoplastic activity of poly(L-lysine) with some ascites tumor cells. PNAS 1979;76:3246-50.

230. Elferink JG. Cytolytic effect of polylysine on rabbit polymorphonuclear leukocytes. Inflammation 1985;9:321-31.

231. De Vries A, Feldman JD, Stein O, Stein Y, Katchalski E. Effects of intravenously administered poly-D L-lysine in rats. Proc Soc Exp Biol Med 1953;82:237-40.

232. Akerberg D, Posaric-Bauden M, Isaksson K, Andersson R, Tingstedt B. Prevention of adhesion by PL/PG after adhesiolysis. J Tissue Sci Eng 2012;3:1-7.

233. Ahmad G, Duffy JM, Farquhar C, et al. Barrier agents for adhesion prevention after gynaecological surgery. Cochrane Database Syst Rev 2008:CD000475.

234. Nehez L, Tingstedt B, Axelsson J, Andersson R. Differently charged polypeptides in the prevention of post-surgical peritoneal adhesions. Scand J Gastroenterol 2007;42:519-23.

235. Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Arch Surg 1974;108:849-53.

236. Bothin CG, Okada M, Midtvedt T. Postsurgical adhesion formation in germfree and ex-germfree rats--a study using three scoring scales. J Invest Surg 1999;12:147-50.

237. Diamond MP, Linsky CB, Cunningham T, Constantine B, diZerega GS, DeCherney AH. A model for sidewall adhesions in the rabbit: reduction by an absorbable barrier. Microsurgery 1987;8:197-200.

238. Zuhlke HV, Lorenz EM, Straub EM, Savvas V. [Pathophysiology and classification of adhesions]. Langenbecks Arch 1990:1009-16.

239. Oncel M, Remzi FH, Senagore AJ, Connor JT, Fazio VW. Comparison of a novel liquid (Adcon-P) and a sodium hyaluronate and carboxymethylcellulose membrane (Seprafilm) in postsurgical adhesion formation in a murine model. Dis Colon Rectum 2003;46:187-91.

240. Lang RA, Weisgerber C, Gruntzig PM, Weis C, Odermatt EK, Kirschner MH. Polyvinyl alcohol gel prevents adhesion re-formation after adhesiolysis in a rabbit model. J Surg Res 2009;153:12-6.

241. Coccolini F, Ansaloni L, Manfredi R, Campanati L, Poiasina E, Bertoli P et al. Peritoneal adhesion index (PAI): proposal of a score for the "ignored iceberg" of medicine and surgery. World J Emerg Surg 2013;8:6.

242. Fang CC, Chou TH, Lin GS, Yen ZS, Lee CC, Chen SC. Peritoneal infusion with cold saline decreased postoperative intra-abdominal adhesion formation. World J Surg 2010;34:721-7.

243. Stewart B, Khanduri P, McCord C, Ohene-Yeboah M, Uranues S, Vega Rivera F et al. Global disease burden of conditions requiring emergency surgery. Br J Surg 2014;101:e9-e22.

244. Ten Broek RP, Kok-Krant N, Bakkum EA, Bleichrodt RP, van Goor H. Different surgical techniques to reduce post-operative adhesion formation: a systematic review and meta-analysis. Hum Reprod Update 2013;19:12-25.

245. Parker MC, Ellis H, Moran BJ, Thompson JN, Wilson MS, Menzies D et al. Postoperative adhesions: ten-year follow-up of 12,584 patients undergoing lower abdominal surgery. Dis Colon Rectum 2001;44:822-29; discussion 9-30.

246. Parker MC, Wilson MS, Menzies D, Sunderland G, Thompson JN, Clark DN et al. Colorectal surgery: the risk and burden of adhesion-related complications. Colorectal Dis 2004;6:506-11.