Outcome and prevention strategies in peritoneal adhesion formation

FANNY FREDRIKSSON
Peritoneal adhesions occur in up to 93% of adults after peritoneal trauma during surgery. Most adhesions are asymptomatic but can cause female infertility, small bowel obstruction (SBO) and chronic abdominal pain. Adhesion prophylaxis is needed to reduce the significant morbidity and increased health care costs resulting from peritoneal adhesions. This thesis aims to establish a relevant and reproducible experimental adhesion model to simultaneously study the healing process and adhesion formation and later to examine whether carbazate-activated polyvinyl alcohol (PVAC), an aldehyde-carbonyl scavenger, can reduce adhesion formation or not; and, in a long-term follow-up, to investigate the incidence of and identify risk factors for adhesive SBO requiring surgical treatment after laparotomy during infancy and to survey the prevalence of self-reported chronic abdominal pain and female infertility. Male Sprague-Dawley rats were subjected to laparotomy, cecal abrasion, and construction of a small bowel anastomosis and examined at various time points after surgery. Early elevation of IL-6, IL-1β and TNF-α concentrations in peritoneal fluid but not in plasma correlate to adhesion formation in this rodent adhesion model, indicating that anti-adhesion treatment should be early, local and not systemic. The animals were treated with either peritoneal instillation of PVAC, or the anastomosis was sutured with PVAC-impregnated resorbable polyglactin sutures. At day 7, bursting pressure of the anastomosis was measured and adhesions were blindly evaluated using Kennedy- and Nair scoring systems. PVAC-impregnated sutures reduced adhesion formation without reducing bursting pressure. Infants who underwent laparotomy between 1976 and 2011 were identified (n=1185) and 898 patients were included with a median follow-up time of 14.7 (range 0.0-36.0) years. The median age at first laparotomy was 6 (range 1.0-365.0) days. There were 113 patients (12.6%) with adhesive SBO, with the highest incidence found in patients with Hirschsprung’s disease (19 of 65, 29%), malrotation (13 of 45, 29%), intestinal atresia (11 of 40, 28%) and necrotizing enterocolitis (16 of 64, 25%). Lengthy duration of surgery (hazard ratio (HR) 1.25, 95% CI, 1.07 to 1.45), stoma formation (HR 1.72, 1.15 to 2.56) and postoperative complications (HR 1.81, 1.12 to 2.92) were independent risk factors. Chronic abdominal pain was reported in 180 (24.0%) of 750 patients, and 17 (13.8%) of 123 women reported infertility. The morbidity after laparotomy in neonates and infants is high. Awareness of the risk factors may promote changes in surgical practice.

Keywords: peritoneal adhesion prevention, inflammatory cytokines, experimental adhesion model, adhesive small bowel obstruction, adhesion-related morbidity

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"Skilled is the physician who is able to prevent or lessen the effects of complications which so often arise in the treatment of wounds."

Paracelsus (1493-1541)

To David, Alexander and Samuel
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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## Abbreviations

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<tbody>
<tr>
<td>ASBO</td>
<td>adhesive small bowel obstruction</td>
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<tr>
<td>BW</td>
<td>body weight</td>
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<tr>
<td>CMC</td>
<td>carboxymethylcellulose</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxygenase</td>
</tr>
<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
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<tr>
<td>DNPH</td>
<td>2,4-dinitrophenylhydrazine</td>
</tr>
<tr>
<td>DNP-hydrazone</td>
<td>2,4-dinitrophenylhydrazone</td>
</tr>
<tr>
<td>ECL</td>
<td>electrochemiluminescence</td>
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<tr>
<td>ECM</td>
<td>extracellular matrix</td>
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<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
</tr>
<tr>
<td>FDA</td>
<td>food and drug administration (government)</td>
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<tr>
<td>GSH</td>
<td>glutathione</td>
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<tr>
<td>GSSG</td>
<td>glutathione disulfide</td>
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<tr>
<td>HA</td>
<td>hyaluronic acid</td>
</tr>
<tr>
<td>HA-CMC</td>
<td>hyaluronate carboxymethylcellulose</td>
</tr>
<tr>
<td>HA-PBS</td>
<td>hyaluronic acid phosphate-buffered saline</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>hematoxylin eosin</td>
</tr>
<tr>
<td>HR</td>
<td>hazard ratio</td>
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<tr>
<td>IFN-γ</td>
<td>interferon gamma</td>
</tr>
<tr>
<td>IL-1,6,10</td>
<td>interleukin-1,6,10</td>
</tr>
<tr>
<td>LOX-1</td>
<td>lectin-like oxidized low density lipoprotein receptor 1</td>
</tr>
<tr>
<td>MDA</td>
<td>malondialdehyde</td>
</tr>
<tr>
<td>MMP-1</td>
<td>matrix metalloproteinase-1</td>
</tr>
<tr>
<td>NADPH oxidase</td>
<td>nicotinamide adenine dinucleotide phosphate-oxidase</td>
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<tr>
<td>NSAID</td>
<td>non-steroidal anti-inflammatory drugs</td>
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<tr>
<td>NO</td>
<td>nitricoxide</td>
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<tr>
<td>PAI</td>
<td>plasminogen activator inhibitor</td>
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<tr>
<td>PDGF</td>
<td>platelet-derived growth factor</td>
</tr>
<tr>
<td>PMNL</td>
<td>polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PTFE</td>
<td>polytetrafluoroethylene</td>
</tr>
<tr>
<td>PVAC</td>
<td>carbazate-activated polyvinyl alcohol</td>
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<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>SBO</td>
<td>small bowel obstruction</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>SOD</td>
<td>superoxide dismutase</td>
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<tr>
<td>TGF-β</td>
<td>transforming growth factor beta</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor alpha</td>
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<tr>
<td>TIMP</td>
<td>tissue inhibitor of metalloproteinases</td>
</tr>
<tr>
<td>tPA</td>
<td>tissue plasminogen activator</td>
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<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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Introduction

Background
The ancient Egyptians described pelvic adhesions as far back as 2500 years ago. Several centuries later, in 440 A.D., pleural adhesions were described in the Babylonian Talmud. But it was not until the development of modern anesthetic techniques in the mid-1800s that the first step towards performing advanced surgery was achieved, thereby increasing the amount of peritoneal adhesions and adhesion-related complications. In 1872, the British surgeon Thomas Bryant described a fatal case of intestinal obstruction caused by peritoneal adhesions that developed after removal of an ovarian tumor. The first published reports describing ways to prevent adhesions began to appear in the surgical literature already in the 1880s, starting with amniotic fluid, bovine cecum, gold-beater's skin (bovine intestinal muscularis and serosa), shark peritoneum, fish bladder and vitreous of calf eyes, which became more refined over the years with the advent of various gums, lubricants, fluids, gels, polymers, physical barriers, all aimed at preventing adhesions (1). This short historical review emphasizes that we have known about adhesions for millennia and that many methods have been employed in an attempt to reduce or prevent the formation of adhesions. And even though our level of understanding the pathogenesis has increased over the years, we still have much to learn in order to diminish the significant burden of adhesions.

Definitions
Peritoneal adhesions are fibrous bands of scar tissue between the peritoneal surfaces of organs and/or tissues in the abdominal cavity that are normally separated. The adhesions are usually found between the greater omentum, loops of bowel (both large and small intestine), liver, spleen, internal female genital organs and the parietal peritoneum (2,3). Adhesions constitute a continuum from a thin film of connective tissue to a thick, highly cellular, vascularized, innervated and dynamic structure, and are formed under the influence of complex signaling pathways (4–6). Adhesions can be either congenital or acquired. Congenital adhesions are created during an abnormal embryologic development of the peritoneal cavity, and exist from birth. Acquired adhesions are subdivided into inflammatory or postoperative. Inflammatory
adhesions arise after inflammatory conditions or peritoneal infections, while most acquired adhesions are postoperative, caused by injury to the peritoneal mesothelium. Postoperative adhesions can be further classified as de novo or reformed. De novo adhesions (type 1) are formed at sites with no previous adhesions at the initial surgery. In contrast, reformed adhesions (type 2) arise at sites where previous adhesiolysis has been performed. According to this classification, the likelihood of postoperative adhesion formation appeared in the following order: adhesiolysis and treatment of pathology (2B) > sites of adhesiolysis alone (2A) > sites of surgical procedures without adhesions (1B) > de novo formation (1A) (7). There is a higher incidence of peritoneal adhesions (67-93%) in patients who have undergone previous surgery than in individuals who have not (10-28%) (2,3). Apart from these studies, it has been difficult to assess the extent of adhesion formation after surgery because a second surgical procedure is needed to detect adhesions. Most adhesions are clinically asymptomatic, however, there is no consensus on how to assess them. Although there have been efforts made to develop a standardized scoring system for adhesion assessment, no scoring system has become the gold standard. Instead, there are a plethora of different scoring systems described in the literature. The ideal scoring system should be simple (easy to remember, apply and analyze), reproducible between both patients and surgeons, and meanwhile versatile enough to consistently demonstrate relevant differences.

The peritoneum

The peritoneum is derived from the mesoderm and its function, to allow free movement by minimizing friction between abdominal viscera, reminds of other serous membranes, e.g. the pleura, pericardium, and tunica vaginalis. With a surface area equal to that of the skin (1.5-2 m²), the peritoneum is the most extensive serous membrane in the body. The visceral peritoneum accounts for about 80% of the total peritoneal surface area and lines the gut and other viscera. The parietal peritoneum, accounting for the remaining 20%, lines the walls of the abdominal cavity (8). The visceral- and parietal layers are separated by a lubricating fluid (glycosaminoglycan and surfactant), which enables peritoneal function (9,10). One of the unique properties of the peritoneum is its delicacy, consisting of a single outer layer of poorly interconnected squamous mesothelial cells (loose intercellular bridges with tight junctions, adherent junctions, gap junctions and desmosomes), which reside on a basal lamina, making the peritoneal surface highly susceptible to trauma (4,11–14). Minimal mobilization of or damage to the peritoneum can result in detachment of the mesothelial cells and denudation of the basal lamina, ultimately causing the formation of adhesions (15). The basal lamina itself is supported by connective tissue with a loose network of collagenous
and elastic fibers, scattered fibroblasts, macrophages, mast cells and varying numbers of fat cells. This layer also provides a capillary network (peritoneal capillaries and lymphatic vessels) with free fluid resorption or diffusion of water and solutes (4,8,11,13,16). The mesothelial cells have microvilli and produce the peritoneal fluid, which contains water, electrolytes, solutes and various cells including leukocytes and macrophages (10). These cells, together with the mesothelium, secrete various cellular mediators that interact in peritoneal healing, by modulating the inflammatory response (4,11). The overall surface of a peritoneal injury becomes covered by mesothelium instantly (and not gradually by ingrowth from the borders as in epidermalization of skin wounds), owing to implantation of free-floating mesothelial cells supported by differentiation of underlying mesenchymal cells, e.g. metaplasia of subperitoneal connective tissue cells and the transformation of peritoneal cells into mesothelial cells (9,15). Peritoneal remesothelialization after trauma is completed within 5-7days, irrespective of the size of injury (17,18).

Pathophysiology

Surgical trauma to the peritoneum can occur by various mechanisms: cutting, abrasion, ischemia, desiccation and coagulation. Regardless of the mechanism, the response of the peritoneum to surgical trauma is similar (11). Surgical injury to the peritoneum induces an excess production of reactive oxygen species (ROS), which in turn cause oxidative stress and damage to the DNA, proteins and cell membranes, e.g. mesothelial cells balloon and detach from the basal lamina, thereby creating denuded areas (14,19–21). Meanwhile, vasoactive substances (i.e. histamine and kinins) are released by the disruption of stromal mast cells. Together with the inflammation related to the oxidative stress, these substances can increase vascular permeability with consequent exudation from the injured surfaces of a fibrinogen-rich fluid, containing histamines, monocytes, plasma cells, polymorphonuclear leukocytes (PMNLs), macrophages, mesothelial cells and histiocytes (4,8,12,21). Meanwhile, activation of the coagulation system results in thrombin formation, which is necessary for the conversion of fibrinogen to fibrin. Fibrin is deposited along injured peritoneal sites, where the fibrin monomer becomes a soluble fibrin polymer. The fibrin polymers interact with fibronectin to form a fibrin gel matrix. The fibrin gel matrix includes leukocytes, erythrocytes, platelets, endothelium, mast cells and cellular debris. Two damaged peritoneal surfaces coming into apposition while covered with fibrin gel matrix may form an adhesion, not only at the time of surgical injury, but also over the following 3-5 days. Under most circumstances, the formation of a fibrin matrix during wound healing is only temporary, and degradation of the fibrin matrix by locally released proteases of the fibrino-
lytic system occurs within 72 hours of injury (4,8,18). Tissue plasminogen activator (tPA) is produced by endothelial cells, mesothelial cells and macrophages. tPA serves to convert plasminogen into active plasmin, which is a broad range protease, capable of degrading fibrin. Plasminogen activator inhibitor (PAI) is produced by similar cell types, i.e. endothelial cells, mesothelial cells, macrophages and fibroblasts. However, PAI inhibits fibrinolysis and facilitate adhesion formation (8,12,22). tPA and PAI-1 levels can be altered by either the type of fibroblast, where adhesion fibroblasts have, in comparison to normal peritoneal fibroblasts, decreased tPA and increased PAI-1, or by the environment. Under hypoxic conditions, both normal peritoneal fibroblasts and adhesion fibroblasts have decreased tPA and increased PAI-1 (23), but this effect is more prominent in the adhesion fibroblasts, along with decreased nitric oxide (NO) levels and increased expression of collagen type 1, fibronectin, MMP-1,TIMP-1,VEGF, α-SM1 actin, COX2, TGF-β1, TGF-β2, IL-10 and IFN-γ (24–26). An inadequate blood supply and reduced tissue oxygenation lead to hypoxia, which stimulates anaerobic metabolism and oxidative stress, which in turn is an interaction between ROS and proteins, lipids, carbohydrates and nucleic acids where ROS modifies the biomolecules into having an altered function, ultimately leading to irreversible cellular damage (Figure 1).
Acute oxidative stress can further induce peritoneal hyperpermeability, mesothelial loss and fibrosis (27). One example of oxidative damage is lipid peroxidation, an oxidative degradation of lipids with a disruption of cellular and membrane integrity, caused by the reaction of ROS with any polyunsaturated fatty acid e.g. arachidonic acid, linoleic acid, eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA). Lipid peroxidation end products are aldehydes, including malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE) and acrolein, which are highly reactive small molecules that covalently bind proteins and DNA, thus altering their functions (28). Oxidized proteins, so-called carbonyls, may further drive an inflammatory reaction by signaling through specific receptors, e.g. lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) (29). A recent study suggests that LOX-I is
involved in the regulation of collagen synthesis, being responsible for cross-linking of collagen fibers (30) (Figure 2).

![Diagram](image)

Figure 2. LOX-1 involvement in collagen synthesis, adapted from (29).

LOX-1 stimulates fibrosis and causes both inflammatory injuries and fibrosis in different organs (31–33). Oxidative stress is a result of the imbalance from both decreased scavenger molecules, e.g. superoxide dismutase (SOD), catalase and glutathione peroxidase, and increased ROS activity, including xanthine oxidase, malondialdehyde and superoxide anions (28). The oxidative stress initiates the formation of the adhesion phenotype and, once developed, it is irreversible, even after restoration of normoxia (24,34). Scavenging of superoxide during hypoxia is the only protection against development of the adhesion phenotype (25). The superoxide also triggers a cascade of inflammatory cytokines with a mesothelial release of tumor necrosis factor-alpha (TNF-α) and interleukin-1 and -6 (IL-1β, IL-6), which reduce the fibrinolytic activity, both by increasing the levels of PAI (12,35–42) and by decreasing the levels of tPA (12,35–40,42,43), thereby shifting the equilibrium between coagulation and fibrinolysis towards coagulation. This imbalance, favoring coagulation in the early phase of peritoneal healing, can promote peritoneal adhesion formation (4,8). PDGF and TGF-β released from plate-
lets recruit and stimulate activation of inflammatory cells from peritoneal vasculature and peritoneal fluid to the injured area (44,45). The injured area is thus invaded by inflammatory cells, initially neutrophil granulocytes that are then, after 24-36 hours, replaced by mostly macrophages (44,46,47). The macrophages multiply and change function in order to secrete cyclooxygenase, lipooxygenase metabolites, tPA, PAI, collagenase, elastase, IL-1 and IL-6, TNF-α, leukotriene, prostaglandins etc. While the main mediators of inflammation-induced activation of coagulation are proinflammatory cytokines (TNF-α, IL-1β, IL-6) the reverse can also occur - the coagulation system can modulate the inflammatory response (40,48). The macrophages recruit new mesothelial cells from adjacent tissue, mesenchymal stem cells and free peritoneal cells to the surface of the injury (13,15,44). In response to cytokines and other macrophage-secreted mediators these mesothelial cells can form cell islands connected by desmosomes and tight junctions, enabling peritoneal remesothelialization to occur (13,15,44). Adhesion formation is the result of both insufficient fibrinolytic capacity and increased fibrin formation in response to an enhanced inflammatory status of the peritoneum (8). The local fibrinolytic capacity is a resultant of the three systems: coagulation, fibrinolysis and inflammation; which is decisive in whether the transitory fibrin matrix is lysed in order for healing by first intention to occur, or whether it is to be further organized by second intention into permanent adhesions (8,11,42–44,49–55) (Figure 3).
The latter alternative, organization into permanent adhesions, involves deposition of an extracellular matrix (ECM; containing fibronectin, hyaluronic acid, proteoglycans, and various glycosaminoglycans) and is directed by and maintained through the action of TGF-β and VEGF-A (44–46,57,58). The

*Figure 3. Flow-chart of adhesion formation, adapted from (8,49,56).*
Temporary fibrin matrix gradually becomes more organized over time as collagen-secreting fibroblasts and other reparative cells infiltrate the matrix. During the first week to the first month after trauma, the fibrous bands transform into highly organized cellular structures that contain arterioles, venules, capillaries (the revascularization is VEGF dependent) and nerve fibers, in addition to collagen (5,11,35,44,46,49,51,52,59,60).

Clinical aspects

Adhesions are considered to be a postoperative complication, in analogy with wound infection or anastomotic leakage, but unlike these other temporary complications, adhesions put patients at lifelong risk of various complications. To make matters worse, it is impossible to predict who will develop clinical symptoms or remain asymptomatic, since performing a second-look procedure merely to evaluate the presence of postoperative adhesions in patients can induce new adhesions and is unethical. Our knowledge is based on readmitted patients with clinical symptoms of adhesive bowel disease that are subjected to relaparotomy. Approximately 1% of all surgical admissions and 3% of laparotomies are due to intestinal obstruction caused by adhesions (3). In a systematic review of adult and pediatric literature, adhesive small bowel obstruction (adhesive SBO) requiring surgical intervention occurred in 2% of all cases after abdominal surgery (61). Overall, the incidence of readmissions directly related to adhesions (treated both surgically and non-surgically) varies from 5-20% in the literature (44,62–64). The mean overall incidence of adhesive SBO in neonates, infants and children in 11 previous studies was 6.2% and ranged from 2.3 to 19.5% (65). There are only three studies on the incidence of adhesive SBO in neonates, reporting 3.3-8.3% (66–68). Making this common problem even more complex, it is, not only the number of adhesions that must be taken into account. The location and structure of adhesions also need to be considered, as these factors can either cause them to remain asymptomatic for years, even decades, or cause early adhesive SBO. In most cases, peritoneal adhesions are clinically asymptomatic but they can become a significant cause of morbidity and even mortality. The total burden of adhesion-related complications and consequences to patients and society has often been underestimated or overlooked in the literature and in clinical practice, since less than 10% of surgeons and gynecologists routinely inform their patients about the risk of adhesion-related complications (69,70). Peritoneal adhesion formation accounts for more than 40% of intestinal obstructions, of which 60-70% involve the small bowel (adhesive SBO), and are associated with mortality rates up to 30% (50,71,72). Adhesions of the fallopian tubes are a leading cause of acquired female infertility, where 20-40% of women have adhesions at laparoscopic evaluation (73–75). However, it is difficult to distinguish their origin as
postsurgical, or caused by pelvic inflammatory disease or by endometriosis. Infertility is a rather common problem in the normal population at 10%, and it is attributed to factors in either the woman (1/3), the man (1/3) or both (1/3) (76). Adhesions are considered as a possible cause of chronic pelvic and abdominal pain (63,77). Whether adhesions themselves are capable of eliciting pain or not is currently uncertain. Sensory nerve fibers have been demonstrated in human peritoneal adhesions, but not all patients with adhesions experience pain (5). A significant problem in evaluating self-reported abdominal pain in relation to adhesions, is defining the prevalence of abdominal pain in the normal population, which has been reported to be 20% (78). In a randomized double-blind controlled study from 2003, 100 women underwent diagnostic laparoscopy for chronic abdominal pain attributed to adhesions. They were randomly assigned either to laparoscopic adhesiolysis or to no treatment. Both groups reported significant pain relief with no difference between the groups, indicating that laparoscopy has a considerable psychological effect and, therefore, laparoscopic adhesiolysis could not be recommended in patients with chronic abdominal pain and adhesions (79). In contrast, at long-term follow-up after a randomized trial comparing an anti-adhesion barrier film with saline controls, a significantly lower incidence of chronic abdominal complaints (including pain) was reported in the anti-adhesion barrier group (80). In addition to adhesive SBO, infertility and chronic abdominal pain, the presence of adhesions often complicates subsequent surgical abdominal procedures, with longer operative times (81,82), increased blood loss and more complications (4,83). Moreover, peritoneal adhesions may cause further morbidity in the form of abdominal discomfort, constipation, impaired growth and reduced appetite, along with more missed school days for the children and time off work for the parents. The significant morbidity and mortality caused by peritoneal adhesions can also be viewed from an economic perspective, with increased operative times and longer hospital stays for adhesive SBO and other adhesion-related problems (61,84–86). In 1994, the estimated financial impact on direct patient care due to adhesion-related disorders in the United States was 1.3 billion USD (85). In Sweden, a study of long-term follow-up of 102 adults with 273 episodes of adhesive SBO showed a cost of 6702 EUR per in-patient episode (87). There are to date no cost analyses of adhesion-related admissions for children. Children’s expected long lifespan and hence the risk of later complications, must also be taken into account. The financial burden of adhesion-related disease will probably also escalate due to improved neonatal care of prematurely born children, to the increasing number of patients requiring surgical care in our aging populations, and to the overall accelerating costs of health care.
Prevention

Over the years, several approaches have been employed to reduce the formation of adhesions and some have been promising (88,89). The difficulty lies in abolishing or reducing the incidence, severity, extent and consequences of adhesions while retaining normal wound- and anastomotic healing. Adhesion formation and healing share the same processes: exudation, coagulation, fibrin deposition, fibroblastic activity and proliferation. Any adhesion prevention should be anti-inflammatory, non-immunogenic and non-infectious. A systemic drug delivery is less likely by the fact that the vulnerable, ischemic sites are cut off from the bloodstream. In addition to being safe and effective, the ideal adhesion prevention should be easy to apply and have a persistent effect during the pivotal remesothelialization phase, approximately 7 days. Both fortunately and unfortunately, the peritoneal membrane has rapid absorptive mechanisms, limiting the half-life and efficacy of peritoneally administered drugs. The main strategies for preventing the formation of peritoneal adhesions are: limiting peritoneal trauma during surgery, removal of foreign-body material from the abdominal cavity, minimal invasively surgery, and application of different fluid- or solid barriers in the abdominal cavity. Limiting peritoneal trauma is accomplished by careful and gentle tissue handling. Other measures include keeping tissues moist with irrigation (but avoiding overheated irrigation fluids), using micro- and non-traumatic instruments and avoiding cautery, retractors, large abdominal wounds, and unnecessary dissection, to reduce serosal injury. It is also crucial to avoid unnecessary activation of the clotting cascade, caused by fibrin-released thromboplastin from ischemic peritoneal tissue and insufficient hemostasis (44,47,49,50,56,90,91). Suturing of peritoneal tissue is considered to increase ischemia and cause devascularization, leading to decreased fibrinolytic activity and increased adhesion formation (92). The suture materials can induce foreign-body reactions. Monofilament sutures are less tissue-reactive than braided sutures, which also contain small pores where bacteria can multiply and cause infections (50). Glove powder (talc or starch) is another foreign-body material that causes peritoneal inflammatory reaction, which potentiates adhesion formation and can also cause foreignbody granulomas (93). When compared with open surgery, laparoscopy can reduce the incidence of de novo-adhesions but not reformation of adhesions, (35,94–97). Other advantages of laparoscopy are smaller skin incision size, earlier return of bowel function and ambulation, less bleeding, less desiccation, less exposure to foreign bodies (such as glove powder and laparotomy pads), shorter hospital stays and the pneumoperitoneum maintained during laparoscopic surgery helps to control hemostasis by the tamponade effect. There are also potential disadvantages associated with laparoscopic procedures, however, such as longer duration of surgery, direct damage to peritoneal mesothelium through a combination of ischemia, acidosis and desiccation.
caused by the carbon dioxide insufflation (98,99). To reduce laparoscopically induced adhesion formation, it is important to survey humidity, and temperature of the peritoneum (100). Since the introduction of laparoscopy in the 1980s, there has been an ongoing debate about whether laparoscopy reduces the incidence of adhesive SBO or not. There are no randomized controlled trials but there is some evidence that laparoscopic surgery is associated with reduced adhesion formation and fewer adhesion-related admissions compared with open surgery (35,94–97,101,102). Even so, adhesion prevention is also necessary in laparoscopic surgery due to the significant adhesion burden (100,103). Adjunct therapy can be directed at one or several of the components of inflammation, coagulation, and fibrinolysis. Drugs that have been tested in this respect, by peritoneal administration, comprise nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, antihistamines, estrogen, anticoagulants, fibrinolytics and antibiotics. No reproducible, significant effects on adhesion formation in patients have been demonstrated. NSAIDs alter arachidonic acid metabolism by inhibiting cyclooxygenase, thereby blocking the formation of prostaglandins and thromboxane. Through this mechanism, NSAIDs reduce vascular permeability, PAI, platelet aggregation and coagulation, and enhance macrophage function. NSAIDs reduce peritoneal adhesion formation in many, but not all, animal models, and their use in human patients is controversial due to the elevated risk of bleeding (104,105). Antihistamines are often used in conjunction with corticosteroids to inhibit fibroblast proliferation. Corticosteroids alone attenuate the inflammatory response by reducing vascular permeability and the release of cytokines and chemotactic factors. Their side effects, however, immunosuppression and delayed wound healing, have made them obsolete as adjuvants (106,107). In animal models, antiestrogen treatment has proven to decrease the formation of peritoneal adhesions, but it is not known whether a hypoestrogenic state renders less adhesions in humans or not (108). Anticoagulants that inhibit fibrin formation are associated with hemorrhages and delayed wound healing (50,109). Fibrinolytics can also cause hemorrhagic complications, although, when applied locally, recombinant tPA reduced adhesions in animal models (110). Antibiotics (cefazolin, tetracycline) in an intra-abdominal irrigation fluid actually caused experimental adhesion formation and have not been recommended for adhesion prevention (111). Barriers separate injured peritoneal surfaces, allowing them to heal without forming fibrinous attachments and later adhesions. The barriers can be in either liquid or solid form. Liquid barrier solutions comprise crystalloids such as Ringer’s lactate, hyaluronic acid (HA), HA in phosphate-buffered saline (Sepracoat®), carboxymethylcellulose (CMC), and icodextrin (Adept®). A crystalloid solution is absorbed too quickly from the peritoneal cavity (in under 24h) long before the fibrin deposition and adhesion formation are complete. Large volumes of fluid in the peritoneal cavity after surgery may also increase the risk of infection (112). HA is a naturally occurring gly-
cosaminoglycan that is water soluble, non-immunogenic and non-toxic, and is a bioabsorbable macromolecule that plays several roles in cellular biology. HA coats serosal surfaces and protects them from injury such as desiccation. However, its use after tissue injury has not proven effective (12,113). HA combined with phosphate-buffered saline (HA-PBS), is applied intraoperatively, prior to dissection, to protect peritoneal surfaces from abrasion and desiccation. In experimental models, it improves peritoneal healing by facilitating cell detachment and migration, and increases the proliferation rate of mesothelial cells, thereby helping to restore denuded areas of the mesothelial lining. In animal models, HA-PBS effectively reduced serosal damage, inflammation and peritoneal adhesions (114). HA-PBS has had only moderate efficacy against the formation of de novo adhesions in human studies, and has to a large extent been abandoned as an anti-adhesion strategy (115). CMC is negatively charged, hydrophilic and freely soluble, and is believed to separate injured peritoneal surfaces, allowing independent healing. CMC has been reported as an anti-adhesion agent in experimental models, but not in the clinical trials performed (44). Icodextrin is a non-viscous, iso-osmotic, clear solution of a glucose polymer that has anti-adhesion effects by separating damaged peritoneal surfaces during remesothelialization via hydroflotation that is not site-specific, and the agent is maintained by a slow resorption (3-4 days) from the peritoneal cavity. It is easily applied during laparoscopic surgery. Several clinical studies have been conducted with mixed results (89,116–120). Complications due to icodextrin instillation in patients have been septicemia, inflammatory states, anastomotic insufficiency and labial swelling (89,116–119,121).

Examples of solid barriers include polytetrafluoroethylene, PTFE (Gore-Tex®), oxidized regenerated cellulose (Interceed®) and bioresorbable membrane of chemically derived sodium hyaluronate and carboxymethyl-cellulose, (HA-CMC; Seprafilm®). They are all difficult to apply during laparoscopic procedures. PTFE is a non-reactive, antithrombogenic, non-toxic, synthetic fabric with small pores that inhibit cellular transmigration and tissue adherence. Unfortunately, PTFE is not bioabsorbable and requires suturing to keep it in place. It must be either left in place permanently or removed surgically, making it less suitable as a barrier to prevent adhesions (122). Regenerated cellulose forms a gel within 8 hours after placement and physically separates adjacent injured surfaces, reducing adhesion formation between these surfaces. It does not require suturing to remain in place. Regenerated cellulose reduces the incidence, extent and severity of postoperative pelvic adhesions but has only been studied in gynecological surgery, in which little bleeding occurs (123). Data from animal studies suggests an impaired effect of regenerated cellulose following contact with blood (124). Therefore, a meticulous hemostasis is necessary before its application. Adhesion formation can even increase if regenerated cellulose is placed in areas
where blood accumulates (e.g. the small pelvis), making it a less suitable strategy (125). HA-CMC is a non-toxic, non-immunogenic, biocompatible material, which turns into a hydrophilic gel approximately 24 hours after placement. HA-CMC sheets are placed at potential sites of adhesion formation at the end of the procedure, just before closure, and provide a protective coating around damaged peritoneal surfaces for up to 7 days, covering the remesothelialization phase. HA-CMC is degraded similarly to HA and is fully resorbed by 28 days (12). There have been several human trials with promising results. There is also evidence that HA-CMC reduces the number of adhesive small bowel obstructions requiring surgical intervention (118). However, serious side effects have limited its use, e.g. when HA-CMC was wrapped around a constructed intestinal anastomosis, there was a significant increase in the number of anastomotic leak-related events (peritonitis, fistula, abscess formation, anastomotic leak and sepsis). Additionally, a higher incidence of pulmonary embolism after deposition of HA-CMC has been reported (118,126–131). To conclude, despite their limitations, as previously described, three of these adhesion barriers are currently FDA approved for clinical use in the United States and approved in Europe: oxidized regenerated cellulose (Interceed®), hyaluronate carboxymethylcellulose, HA-CMC (Seprafilm®) and icodextrin (Adept®). They are seldom applied, however, which may explain why a recent survey based on US hospital discharges showed no changes in overall rates of adhesion-related complications (132). So even though there is evidence that barriers (oxidized regenerated cellulose and HA-CMC) reduce adhesion formation (118), the question remains as to whether this reduction will be enough to diminish the significant burden of adhesions.
Aims

General Aims of the Thesis
To establish a relevant and reproducible experimental adhesion model that enables studies of adhesion formation and the healing process simultaneously and, later, to examine the effects of potential anti-adhesion strategies in the model.
To perform a long-term follow-up of peritoneal adhesion-related morbidity following laparotomy during infancy, since robust data on adhesion-related morbidity after laparotomy during infancy is scarce in the literature.

Specific Aims of the Thesis
I: To measure the concentrations in plasma and peritoneal fluid of certain cytokines, proteins and growth factors that promote peritoneal adhesions in an experimental rodent model before and after induction of adhesions.
II: To demonstrate the presence of aldehydes e.g malondialdehyde (MDA) and the LOX-1 receptor in an experimental adhesion model. Furthermore, to investigate whether carbazate-activated polyvinyl alcohol (PVAC), an aldehyde-carbonyl scavenger, can reduce adhesion formation.
III: To investigate the incidence of and identify risk factors for adhesive SBO requiring surgical treatment after laparotomy during infancy and to survey the prevalence of self-reported chronic abdominal pain and female infertility.
Materials and Methods

The most important methodology used in the thesis is described in brief below. Further details can be found in the individual papers.

Animals (I-II)
Outbred male Sprague-Dawley rats were purchased from Taconic (Bomholt, Denmark). The total number of rats used was 178 (52 in paper I and 126 in paper II). They were housed for one week in the animal department at Uppsala Biomedical Center before treatment, where they were maintained under standard laboratory conditions (+22°C, with a 12-hour light/dark cycle, and fed pellet food and water ad libitum). Preoperative body weight (BW) was 337.8 ± 2.3 g (Paper I) and 257 ±2.2 g (Paper II).

Surgical procedure (I-II)
Anesthesia was induced in a sealed chamber with inhaled 4% isoflurane and maintained using a facemask delivering 2.5% isoflurane. All procedures were performed under clean but non-sterile conditions, by one and the same surgeon. Firstly, the abdominal fur was moistened with 70% ethanol and a 3-cm midline abdominal incision was performed. Secondly, the cecum was delivered and abraded with surgical gauze for 2 minutes. The abraded cecum with punctate bleedings was then left outside the abdominal cavity to desiccate. Thirdly, a 1-cm small bowel resection approximately 15 cm from the ileocecal junction was made and a single-layer end-to-end anastomosis was constructed with 8 stitches, using interrupted resorbable 6-0 Monosyn® sutures (B. Braun Medical AB, Danderyd, Sweden) in Paper I, and 6-0 PVAC/Saline impregnated resorbable polyglactin sutures in Paper II. Anastomotic patency was confirmed by feeding small bowel content through it. Fourthly, the cecum and the small bowel were returned to the abdominal cavity in their proper anatomic positions. Finally, the laparotomy incision was closed in 2 layers with continuous resorbable 5-0 Vicryl® sutures (Johnson & Johnson AB, Sollentuna, Sweden) for the fascia in Paper I, and replaced by 5-0 PVAC/Saline-impregnated resorbable polyglactin sutures in Paper II. In both cases, continuous non-resorbable 4-0 Ethilon® sutures
(Johnson & Johnson AB) were used for the skin. The surgical procedure took ~30 minutes. As soon as surgery was finished, the rats were placed in a warm environment for postoperative recovery. Postoperatively, the rats received 0.015 mg buprenorphine (0.3mg/ml) s.c. every 8 hours for 2 days.

Scoring of adhesions (I-II)

An inverted U-shaped abdominal incision was performed to avoid disturbing any adhesions between viscera and the abdominal wall. Macroscopic adhesions were evaluated in a blinded manner by 2 observers using 3 different scoring systems in Paper I, and 2 scoring systems in Paper II (see Table 1) (110,133,134).

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>Adhesion scoring systems.</td>
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<table>
<thead>
<tr>
<th>Menzies</th>
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<tbody>
<tr>
<td>1 = separated with gravity</td>
</tr>
<tr>
<td>2 = separated by blunt dissection</td>
</tr>
<tr>
<td>3 = separated by sharp dissection</td>
</tr>
<tr>
<td>4 = difficult sharp dissection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kennedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 = no adhesions</td>
</tr>
<tr>
<td>1 = thin, filmy adhesions</td>
</tr>
<tr>
<td>2 = &gt; one thin adhesion</td>
</tr>
<tr>
<td>3 = thick adhesion with focal point</td>
</tr>
<tr>
<td>4 = thick adhesion with planar attachment</td>
</tr>
<tr>
<td>5 = very thick vascularized adhesions/&gt;1 planar adhesion</td>
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<table>
<thead>
<tr>
<th>Nair</th>
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<tbody>
<tr>
<td>0 = complete absence of adhesions</td>
</tr>
<tr>
<td>1 = single band of adhesions between viscera/abdominal wall</td>
</tr>
<tr>
<td>2 = two bands between viscera/abdominal wall</td>
</tr>
<tr>
<td>3 = &gt;2 bands between viscera/abdominal wall or whole intestines forming a mass without being adherent to abdominal wall</td>
</tr>
<tr>
<td>4 = viscera adherent to abdominal wall irrespective of number or extent of bands</td>
</tr>
</tbody>
</table>
Anastomotic bursting pressure (I-II)

The bursting pressures of the anastomoses were tested at different time points (72 h, 7 d and 21 d) in Paper I and after 7 days in Paper II, by continuous infusion of Ringer’s acetate in an approximately 4-cm ileal segment containing the anastomosis and ligated at both ends. Bursting pressure was defined as the maximal pressure reached (in mmHg) until leakage from or burst of the anastomosis occurred.

Plasma and peritoneal fluid sampling (I-II)

Blood samples were obtained by intracardiac puncture using a 22 G sterile needle and 5 ml syringes. Samples were transferred to pre-chilled centrifuge vials coated with heparin and spun within 30 minutes at 4000g for 10 minutes at 4°C. The plasma was aliquoted and stored at −80°C until analysis. In Paper I peritoneal fluid samples were collected by peritoneal lavage with 40 ml of Ringer’s acetate; in Paper II the volume was reduced to 10ml. 10 out of 40 ml and 5 out of 10 ml, respectively, of aspirated fluid was snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Morphology (II)

Tissue from the anastomotic site was retrieved and fixed in 4% paraformaldehyde solution. The samples were dehydrated and embedded in paraffin blocks, whereafter sections of 4-5μm thickness were cut, rehydrated and stained with hematoxylin and eosin (H&E). A pathologist blinded to the groups examined the sections under a light microscope and quantified the inflammatory cells.

Enzyme-linked immunosorbent assay (I-II)

ELISA technology is based on the use of antibodies, an enzyme and a chromogen to detect an antigen. The basic steps include to prepare a surface to which the capture antibodies are bound, the sample with antigen is added and captured by the antibodies. The plate is then washed to remove unbound antigen, and another specific antibody is applied, which binds to the antigen, enzyme-linked secondary antibodies are added as detection antibodies, followed by a wash to remove unbound antibody-enzyme conjugates, and finally the enzyme converts the chromogenic chemical into a colored product. The absorbance signal of the assay wells is measured to quantify the antigen, and the optical density of the sample can usually be compared with
a standard curve. The sensitivity of the assay depends on the amplification of the signal during the analytic reactions. The performance of the ELISA further depends on the antibody specificity and avidity and the person running the test. Multiplex kits are based on the same technology but enable measurement of multiple cytokines in the same sample at the same time. This means multiplex kits have multiple specific antibodies coated at corresponding spots on an electric wired microplate. The detection antibody binds to a proprietary tag, which generates emission beams in an electric field. An amplification of the signal is carried out by the "read buffer", a coreactant to the proprietary. In Paper I, the concentrations of cytokines IL-1β, IL-6 and TNF-α were measured in plasma and peritoneal fluid by electrochemoluminescence (ECL) using the Multi Spot®4-Spot Cytokine Plate, Rat 3-plex (Meso Scale Discovery, Rockville, MD) and the detection limits were 2.4 pg/ml for IL-1β, 16.7 pg/ml for IL-6 and 3.1 pg/ml for TNF-α in plasma and 3.1 pg/ml, 0.5 pg/ml and 2.8 pg/ml, respectively, in peritoneal fluid. PDGF-BB, TGF-β1 and VEGF concentrations were measured in peritoneal fluid by quantitative sandwich enzyme immunoassay technique: Quantikine® ELISA Rat PDGF-BB, Quantikine®ELISA Rat TGF-β1 and Quantikine® ELISA Rat VEGF were all obtained from R&D Systems (Abingdon, UK). The detection limits were 7.7 pg/ml for PDGF-BB, 4.6 pg/ml for TGF-β1 and 8.4 pg/ml for VEGF. The Rat Tissue-type Plasminogen Activator (tPA) Active ELISA Assay kit (Eagle Biosciences Inc., Nashua, NH) was used to measure active tPA in peritoneal fluid samples. The detection limit was 50 pg/ml. Active PAI-1 was determined in peritoneal fluid by Zymutest PAI-1 Activity ELISA (Aniara Diagnostica, West Chester, OH) and the detection limit was 100 pg/ml. In Paper II, the concentrations of the cytokines IL-1β and IL-6 were measured in peritoneal fluid by ECL using the Multi-Spot® 4-Spot Cytokine Plate, Rat 3-plex (Meso Scale Discovery, Rockville, MD) and the detection limits were 3.1 pg/ml for IL-1 β and 0.5 pg/ml for IL-6 in peritoneal fluid.

Thiobarbituric Acid Reactive Substances Assay (II)

The OxiSelect™ Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit (Cell Biolabs, Inc, San Diego, CA) was used for direct quantitative measurement of the lipid peroxidation end product malondialdehyde (MDA) in peritoneal fluid. The peritoneal fluid samples or MDA standards (used as positive control) are first reacted with TBA at 95°C. After a brief incubation, the samples and standards were read either spectrophotometrically or fluorometrically. The MDA content in the peritoneal fluid samples was determined by comparison with the predetermined MDA standard curve.
**Immunohistochemistry staining (II)**

The formaldehyde-fixed and paraffin-embedded tissue sections from the anastomotic site underwent deparaffinization in xylene and then rehydration in graded alcohols. Sections were heated in Tris-ethylenediaminetetraacetic acid buffer, pH9, with a PT-link (Dako, Glostrup Denmark) for antigen retrieval. The Dako Autostainer Plus was utilized for immunohistochemical staining, using the rabbit polyclonal anti-rat LOX-1 (ab60178, Abcam, Cambridge, MA) as primary antibody, dilution 1:500.

**Western blot (II)**

80 µg of human albumin (Sigma) was treated with 25 µL of 0.2 µM acrolein (Sigma) for 8 hours at 22°C. Protein oxidation involves the introduction of carbonyl groups into protein side chains. These carbonyl groups will react with 2,4-dinitrophenylhydrazine (DNPH) to produce 2,4-dinitrophenylhydrazone (DNP-hydrazone) (Oxyblot Protein Oxidation Detection Kit, EMD Millipore, Merck KGaA, Darmstadt, Germany). The oxidized proteins were separated on 12% polyacrylamide gels, transferred to polyvinylidene difluoride membrane (Hybond-P, GE Health Care, Uppsala, Sweden) and Western blot was performed using anti-DNP antibodies followed by HRP-labelled secondary antibody and visualized by ECL. Solutions of PVAC (DS 12.7%) with carbazate concentrations of 1 mM and 10 mM were prepared and mixed with albumin prior to adding acrolein to the samples.

**Study population (III)**

Patients who underwent laparotomy during the first year of life in a tertiary pediatric surgical center in Uppsala, Sweden, from 1976 to 2011, were identified. Patients aged 18 years or more, and parents of patients younger than 18 years, received written information about the study and provided written informed consent to participate. Parameters retrieved from the patient’s medical records were e.g. date of birth, gestational age, sex, birthweight, date at initial surgery, end date (the receiving date for each questionnaire/letter), occurrence of adhesive SBO confirmed at relaparotomy caused by peritoneal adhesions, diagnosis, stoma formation, duration of surgery, timing of surgery (office hours or not), significant postoperative complications (wound infection, wound dehiscence, abdominal abscess formation, anastomotic leakage and sepsis) and the total number of laparotomies to which the patient had been subjected. Patients in the study population were sent a questionnaire (non-validated) designed specifically for this study, asking for in-
formation on chronic abdominal pain, hospitalization for abdominal pain and, in women aged 18 years or older, infertility.

Statistical methods (I-III)

In Paper I, cytokine samples and standards were assayed in duplicate and are expressed as means ± standard error of the mean (SEM). Cytokine levels, adhesion scores and anastomotic bursting pressures measured at designated time points were compared using the non-parametric Kruskal-Wallis test and Dunn's Multiple Comparison Test in GraphPad Prism™ version 5.0c.

In Paper II, compared weight differences, adhesion scores, correlation, anastomotic bursting pressures and cytokines using the non-parametric Kruskal-Wallis test, Dunn’s Multiple Comparison Test, correlation test, column statistics and the Mann-Whitney's test in GraphPad Prism™. For both papers I and II, differences of \( p < 0.05 \) were considered statistically significant; \* denotes \( p < 0.05 \), ** denotes \( p < 0.01 \) and *** denotes \( p < 0.001 \). In Paper III, all statistical analyses were performed using R version 3.1.1 (www.R-project.org). Descriptive statistics are presented as median (range) for continuous variables and as absolute and relative frequencies for categorical variables. Time to adhesive SBO is presented as a Kaplan–Meier estimate. All patients were included in the Kaplan–Meier estimate, regardless of the duration of follow-up, which was calculated from the first laparotomy to one of three endpoints: first relaparotomy for adhesive SBO, death, or end-date of the observational period. Possible independent risk factors for developing adhesive SBO were analysed using a Cox regression model and presented as hazard ratios (HRs) with 95% CI. Differences of \( p < 0.05 \) were considered significant but, as no adjustments was made for multiplicity, the \( p \)-values should be interpreted as exploratory. The number of previous operations was excluded from the multivariable analysis because of different lengths of follow-up. Patients who had died were included in all analyses except the risk analysis, because of short follow-up time. The \( \chi^2 \) test was used to compare long-term morbidity in patients who had surgery for adhesive SBO and those who did not.

Ethical considerations (I-III)

The studies underlying papers I and II were approved by the Regional Animal Ethics Committee, and performed in accordance with the National Guide for the Care and use of Laboratory Animals published by the National Research Council in 1996.

The study underlying Paper III was approved by the Central Ethical Review Board in Uppsala (registration number 2010/417).
Results

Paper I

49 of the 52 rats completed the study. Two rats died during anesthesia and one rat died on postoperative day 14 due to small bowel obstruction, confirmed by autopsy. There were no congenital adhesions at the initial laparotomy. Plasma concentrations of cytokines IL-1β and TNF-α were not increased (data not shown). Plasma and peritoneal fluid concentrations of IL-6 peaked at T = 6 hours (138.9 ± 25.4 pg/ml, 6772 ± 1894 pg/ml, respectively). Concentrations of IL-6 decreased significantly at T = 7 days (p < 0.05) in plasma (Figure 4) and at T = 72 hours (p < 0.01) in peritoneal fluid (Figure 5).

Figure 4. IL-6 concentrations + SEM in plasma at different time points after incision.
Figure 5. IL-6 concentrations + SEM in peritoneal fluid at different time points after incision.

Peritoneal fluid concentrations of IL-1β peaked at T = 6 hours (393.4 ± 202.8 pg/ml) and decreased at T = 21 days (p < 0.05) (Figure 6). Peritoneal fluid concentrations of TNF-α peaked at T = 6 hours (21.4 ± 5.8 pg/ml) and remained increased to T = 7 days (p < 0.05) (Figure 7). Peritoneal fluid concentrations of PDGF-BB, TGF-β1, VEGF, tPA and PAI-1 were below the detection levels (data not shown).
Figure 6. IL-1β concentrations ± SEM in peritoneal fluid at different time points after incision.

Figure 7. TNF-α concentrations ± SEM in peritoneal fluid at different time points after incision.
Menzies scoring system (Figure 8) revealed significantly higher scores at T = 7 days (2.9 ± 0.4) and T = 21 days (4.0 ± 0.0) compared to earlier time points. The Kennedy scoring system (Figure 9) revealed significantly higher scores at T = 24 hours (3.9 ± 0.1) and at T = 21 days (5.0 ± 0.0) compared to earlier time points. Nair scoring (Figure 10) revealed higher scores at T = 24 hours (3.1 ± 0.1) and at T = 7 days (3.1 ± 0.4) compared to earlier time points. Anastomotic bursting pressures were significantly increased at T = 21 days (292 ± 48.7) compared to T = 72 hours (52.5 ± 5.0).

Figure 8. Menzies score at different time points after incision.
Figure 9. Kennedy score at different time points after incision.

Figure 10. Nair score at different time points after incision.
The Western blots show diminished protein oxidation in PVAC-treated samples as compared to controls and the effect was dose-dependent (Figure 11).

<table>
<thead>
<tr>
<th></th>
<th>Acrolein</th>
<th>Albumin (4mg/ml)</th>
<th>PBS</th>
<th>PVAC</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neg.control</td>
<td></td>
<td>20 µl</td>
<td>30 µl</td>
<td>-</td>
<td>w/o DNPH</td>
</tr>
<tr>
<td>Lane 1</td>
<td></td>
<td>20 µl</td>
<td>30 µl</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lane 2</td>
<td>25 µl, 20 µl</td>
<td>20 µl</td>
<td>5 µl</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lane 3</td>
<td>25 µl, 20 µl</td>
<td>20 µl</td>
<td>-</td>
<td>5 µl, 1mM</td>
<td></td>
</tr>
<tr>
<td>Lane 4</td>
<td>25 µl, 20 µl</td>
<td>20 µl</td>
<td>-</td>
<td>5 µl, 10 mM</td>
<td></td>
</tr>
</tbody>
</table>

Figure 11. The effect of PVAC on acrolein-induced protein oxidation in vitro. The Western blot show a dose-dependent diminished protein oxidation in PVAC treated samples as compared to controls.

110 of 126 rats completed the study. Sixteen rats developed signs of intestinal obstruction with extensive weight loss and/or abdominal distension and were euthanized early (postoperative days 2-4) in accordance with the ethical guidelines. No congenital adhesions were noted at the initial laparotomy. There were no differences in BW changes or anastomotic bursting pressures in the various groups. In all groups adhesions were predominantly located at the anastomotic site. The agreement between the blinded observers scoring the adhesions was 0.912. There was no significant difference between peri-
toneal instillation of PVAC or saline according to Nair and Kennedy scoring (data not shown). When using impregnated sutures and evaluating with the Kennedy scoring system, however, a significant difference was seen for the lowest concentration (PVAC 2.5mg/ml) compared to saline with the Mann-Whitney test (p = 0.0406). No significant difference was found between any of the three PVAC concentrations and saline according to the Nair scoring system. The concentrations of IL-1β and IL-6 in peritoneal fluid increased significantly at 6hours compared to the earlier time point (0h) for both PVAC- and saline-impregnated sutures. PVAC IL-1β: 2268 ± 321.6 pg/ml and IL-6: 20851± 1978 pg/ml (p < 0.001), saline IL-1β: 2595 ± 345.2 pg/ml and IL-6: 23491± 1936 pg/ml (p < 0.001). There was no difference in cytokine levels at 6hours between PVAC- and saline-impregnated sutures (figures 12 and 13).

**Figure 12.** Concentrations of IL-1β in peritoneal fluid for PVAC- and saline-impregnated sutures at different time points.
Concentrations of IL-6 in peritoneal fluid for PVAC- and saline-impregnated sutures at different time points.

The concentration of malondialdehyde in peritoneal fluid increased significantly at 6 hours compared to the earlier time point (0h) for both PVAC- and saline-impregnated sutures. PVAC: 0.09533 ± 0.006640 μM (p < 0.0001) and saline 0.1127 ± 0.02020 μM (p < 0.0001). There was no difference at 6 hours between PVAC- and saline-impregnated sutures (Figure 14).
Figure 14. The concentration of the lipid peroxidation product, malondialdehyde (MDA), measured in peritoneal fluid at different time points.

Histopathology showed no significant difference in the number of inflammatory cells between the two groups. Immunohistochemistry showed a positive staining for LOX-1 both in the PVAC-suture and control group (Figure 15).
Figure 15. LOX-1 immunoreactivity was located in a majority of the cells (epithelial, endothelial, muscle and inflammatory cells) in tissue from the anastomotic site. The immunoreactivity was mainly cytoplasmic, indicating a rapid turnover. Negative control (omittance of the primary antibody) below.
Paper III

During the study interval from 1976 to 2011, 1185 patients were eligible for inclusion. Of these, 143 had died and 25 patients could not be reached (registered at the wrong address, emigrated or protected identity). The remaining 1017 patients were sent an invitation to participate in the study. 755 patients (74.2%) agreed to participate and completed questionnaires were received from 750. An amendment to the ethics board approval made it possible to review the medical charts of the deceased patients in order to identify episodes of adhesive SBO as well as cause of death. The total study population was thus 898 patients (Figure 16).

![Flow diagram of the study population](image)

*Figure 16. Flow diagram of the study population.*

The proportion of patients with each specific diagnosis was similar for non-participants and participants, except for pyloric stenosis, where there were significantly more patients with pyloric stenosis among the non-participants (66 of 262 (25.2%) versus 151 of 898 participants (16.8%); p = 0.002). Characteristics of the patients included in the study are shown in Table 2. Most patients (749 of 875, 85.6%) were born between gestational week 33 and term, and the majority of laparotomies were in neonates, at a median age of 6.0 days. There were 113 patients (12.6%) with first-time adhesive SBO requiring relaparotomy during the study period. 62 of these 113 patients (54.9%) had an adhesive SBO following one previous laparotomy. Multiple laparotomies were performed in 51 patients (36 patients had 2, 9 had 3, 4 had 4, 1 had 5, and 1 had 6) before adhesive SBO occurred.
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>No. of patients* (n = 898)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex ratio (M : F)</td>
<td>532 : 366</td>
</tr>
<tr>
<td>Gestational age (weeks) (n = 875)</td>
<td></td>
</tr>
<tr>
<td>25–28</td>
<td>56 (6.4)</td>
</tr>
<tr>
<td>29–32</td>
<td>70 (8.0)</td>
</tr>
<tr>
<td>33 to term</td>
<td>749 (85.6)</td>
</tr>
<tr>
<td>Age at initial operation (days)†</td>
<td>6.0 (1.0–365.0)</td>
</tr>
<tr>
<td>Surgery for adhesive SBO</td>
<td>113 (12.6)</td>
</tr>
<tr>
<td>Death</td>
<td>143 (15.9)</td>
</tr>
<tr>
<td>Duration of follow-up (years)‡</td>
<td>14.7 (0.0‡ to 36.0)</td>
</tr>
</tbody>
</table>

Table 1. Patient characteristics

The interval from the first laparotomy to relaparotomy for adhesive SBO is shown as a Kaplan–Meier estimate in Figure 17.

Figure 17. Kaplan–Meier estimates of the cumulative risk of adhesive small bowel obstruction (SBO) requiring relaparotomy in all patients for up to 20 years.

Overall, the incidence of adhesive SBO was 113 (12.6%), of which 79 (69.9%) occurred within 2 years after the initial laparotomy. One patient, however, had an episode of adhesive SBO as late as 28 years after laparotomy during infancy. Of the 143 patients who died, three deaths were a direct consequence of adhesive SBO. Autopsies were not performed on all deceased patients, but sepsis, liver failure due to short bowel syndrome and congenital heart malformation were stated causes of death. Diagnoses at initial laparotomy according to falling cumulative incidence of adhesive SBO are listed in Table 3. Four diagnoses were associated with a high incidence of adhesive SBO: Hirschsprung’s disease (19 of 65 patients, 29%), malrotation (13 of 45, 29%), intestinal atresia (11 of 40, 28%) and necrotizing enterocolitis (16 of 64, 25%).
Table 2. Diagnosis at initial laparotomy by incidence of surgically treated adhesive small bowel obstruction.

The diagnoses were evenly distributed over the study period, except for patients with necrotizing enterocolitis of whom a majority (53 of 64, 83%) were born in the later time interval, 1991–2011. Stoma formation, duration of surgery greater than 1 h, and postoperative complications, were identified as independent risk factors for development of adhesive SBO (Table 4). For every 1 hour of surgery, the risk of adhesive SBO increased by 25% (HR 1.25).

Table 3. Cox regression analysis of risk factors for the development of adhesive small bowel obstruction.
For the 4 most common diagnoses related to adhesive SBO, the 2-year interval from the first laparotomy is illustrated in Figure 18.

![Graph illustrating the cumulative risk of adhesive small bowel obstruction (SBO) requiring relaparotomy in patients with a high-risk diagnosis during the first 2 years after initial laparotomy.](image)

**Figure 18.** Kaplan–Meier estimates of the cumulative risk of adhesive small bowel obstruction (SBO) requiring relaparotomy in patients with a high-risk diagnosis during the first 2 years after initial laparotomy.

The incidence of adhesive SBO did not differ between patients undergoing first laparotomy in 1976–1990 and those having initial surgery in 1991–2011 (43 of 358; 12.0%, versus 70 of 540; 13.0%, respectively; \( p = 0.171 \)).

In total, 750 patients (297 women and 453 men) answered the questionnaire. Chronic abdominal pain was reported more commonly by women than by men (87; 29.3%, versus 93; 20.5%, \( p = 0.006 \)), as was hospitalization due to abdominal pain (95; 32.0%, versus 87; 19.2%, respectively; \( p = 0.002 \)). Of the 750 patients who completed the questionnaire, 102 had surgery for adhesive SBO. These patients had a higher prevalence of chronic abdominal pain than the 648 patients who did not have adhesive SBO (52; 51.0%, versus 128; 19.8%, respectively; \( p < 0.001 \)), as well as hospitalization for abdominal pain (77; 75.5%, versus 105; 16.2%, \( p < 0.001 \)). Infertility was reported in 17 (13.8%) of 123 women.
Discussion

Adhesion induction by abrasion of the cecal surface and construction of a small bowel anastomosis has previously been described in rabbits (135). Using the more convenient laboratory rat provides a relevant and reproducible experimental model designed for dissection of molecular mechanisms as well as interventional procedures. Combining adhesion induction with construction of an intestinal anastomosis enables study of the balance between anastomotic healing and the prevention of adhesions after anti-adhesion treatment. Anti-adhesion treatment should reduce adhesions without impaired anastomotic healing. We found that IL-1β concentrations were increased early after surgery. Early elevation and high concentrations of IL-1 in peritoneal fluid after surgery are regarded as a biological marker for postoperative adhesions in humans (136,137). Peritoneal injection of IL-1 in rats increased adhesion scores after peritoneal injury (138). IL-1 is secreted by macrophages, and following caspase activation it promotes inflammation and coagulation, and reduces fibrin degradation locally (11). Thus, interference with IL-1β signaling may reduce adhesion formation. We also found increased IL-6 levels in peritoneal fluid and plasma at 6 hours after adhesion induction. This is in accordance with a report of IL-6 increase in serum in six patients within 1.5 hours after abdominal incision, reaching a maximum between 1.5–4 hours. Data suggest that tissue damage may be proportional to IL-6 concentrations (139). Elevated IL-6 at 12- and 24 hours were also seen in our study, as have been reported previously in humans after major abdominal surgery (137). IL-6 is produced by T-lymphocytes, macrophages and fibroblasts at sites of tissue damage (139). It triggers the acute phase response and stimulates mesothelial release of PAI-1 in vitro (37), which may explain the decrease in peritoneal fibrinolytic activity reported at 48–72 hours after surgery in humans (41). Preoperative administration of IL-6 into the peritoneal cavity of rats enhanced adhesion formation and caused more vascular adhesions with a higher number of inflammatory cells and fibroblast deposits. Concentrations of IL-6 in both plasma and peritoneal fluid correlated with adhesions scores following a defined bowel injury in rabbits (135). In summary, our data and that of others suggest that interference with IL-6 signaling may also be an anti-adhesion strategy. TNF-α concentrations were elevated in peritoneal fluid in our study at 6 hours after adhesion induction. TNF-α is a cytokine secreted by several different cell types. It stimulates adhesion formation by promoting the inflammatory re-
sponse and coagulation, and impairs fibrinolysis. Significant correlations between the severity of adhesions and postoperative concentrations of TNF-α in plasma and peritoneal fluid have been reported in both rats and humans (135,136,140). Our data confirm that early elevation of TNF-α concentrations in peritoneal fluid correlates to adhesion formation in rats, and it may also be a therapeutic target. Surprisingly, peritoneal fluid concentrations of PDGF-BB, TGF-β1, VEGF, tPA and PAI-1 were below detection levels. This may reflect either that they do not participate, are not secreted to the peritoneal fluid compartment, are active at later time points, or that the detection limit of our assay was too high (peritoneal fluid was diluted with 40 ml Ringer’s acetate in order to retrieve a sufficient volume for analysis). Adhesion quantification is mandatory for the development of anti-adhesion treatment. The lack of a gold standard forced us to employ three different scoring systems with the intention to identify the most relevant and reproducible system. Our data confirm that the three scoring systems have similar properties, but the most relevant and reproducible system remains to be determined.

Although numerous anti-adhesive barriers (films, gels, sprays, and fluids) and pharmacological therapies (anti-inflammatories, antibiotics, antioxidants, anticoagulants and fibrinolitics) have been proposed, few are used clinically. Barriers of oxidized regenerated cellulose (Interceed®) and hyaluronate carboxymethylcellulose (Seprafilm®) have proved to reduce adhesion formation in clinical trials and, together with the glucose polymer icodextrin (Adept®), are currently approved for clinical use in the United States and Europe (21,88,118,141). They are seldomly used, however, for various reasons, which may explain why one recent survey found no decrease in adhesion-related complications (132). New strategies for preventing adhesion formation are therefore needed. In another recent study, redox injectable gel, an agent with anti-oxidative stress function (a ROS scavenger), successfully reduced adhesion formation in a murine model (142). We applied a new anti-adhesive approach using resorbable sutures impregnated with an aldehyde-carbonyl scavenger and found a significant reduction in peritoneal adhesions without any adverse effects on healing of the small bowel anastomosis or the inflammatory response. The lipid peroxidation end product MDA was detected in peritoneal fluid at 6 hours. This finding is in accordance with increased tissue level of MDA in a rodent adhesion model with only cecal abrasion (143). LOX-1 is a type II transmembrane receptor belonging to the C-type lectin family. It is the first member of the class E scavenger receptor subfamily. Deletion of LOX-1 reduced collagen deposition in an ischemic heart animal model (29). In our model, the LOX-1 receptor was detected in many cells at the anastomotic site, and the presence of LOX-1 indicates that it is available for activation by carbonyls formed after peritoneal injury. After peritoneal instillation of PVAC, there was no significant reduction of
adhesion formation. At autopsy, the majority of adhesions were located at the anastomotic site and not at the abraded cecum. We hypothesized that the anti-adhesive agent would act more locally in the wound and decided to use a different strategy, focusing on the sutures used for construction of the anastomosis. Sutures are foreign-body material, which induce adhesions (50), but they are necessary in construction of an anastomosis and laparotomy closure. Modifying sutures by giving them antibacterial properties has been effective in infection prevention and the healing of wounds (144). In analogy, we impregnated our sutures with PVAC, which is a new approach targeting the foreign body and the first time for using an aldehyde-carbonyl scavenger in peritoneal adhesion reduction. PVAC 2.5mg/ml significantly reduced peritoneal adhesions without interfering with the inflammatory response. The concentrations of IL-1β, IL-6, and MDA in peritoneal fluid increased significantly at 6 hours compared to 0 hours for both PVAC- and saline-impregnated sutures, but there was no difference between PVAC- and saline-impregnated sutures at 6 hours. A possible explanation to why the anti-adhesive effect of PVAC was not mirrored by decreased concentrations of IL-1β, IL-6, or MDA in peritoneal fluid may be that its action is restricted to the site of anastomosis. This assumption is supported by the fact that peritoneal instillation of PVAC did not have an anti-adhesive effect. The strengths of this study are the double-blinded study design with a high correlation between independent observers at scoring, and the use of a relevant and reproducible adhesion model: some rats (16/96, 17%) developed small bowel obstruction, and the construction of a small bowel anastomosis permitted study of the balance between anastomotic healing and adhesion prevention. Experimental studies based on either the ischemic button model or controlled injury to the abdominal wall where adhesions are quantified as the percentage of the injured area covered by adhesions render robust interval variables, while our adhesion model renders ordinal variables but represent a more clinical setting. Because this model is stringent it has been difficult to achieve adhesion reduction when using different potential anti-adhesion strategies in pilot studies (data not shown). One reason and main weakness of our study is the lack of a gold standard in adhesion scoring, and two different scores were used, each with its own merits. Kennedy describes the severity of the adhesions rather than the localization of the adhesions (Nair). The PVAC impregnated sutures have a local effect in reducing adhesions and their severity at the anastomotic site, which might explain why only Kennedy was able to demonstrate significant differences in adhesion scores. Another weakness is that it is a rodent model, and it is not known if our results can be translated to patients.

In our long-term follow-up study we found a high incidence of adhesive SBO after laparotomy during infancy where infants with Hirschsprung’s disease, malrotation, intestinal atresia and necrotizing enterocolitis exhibited
the highest incidences. Independent risk factors for the development of adhesive SBO requiring relaparotomy were long duration of surgery, stoma formation and postoperative complications. Patients who underwent surgery for adhesive SBO had a significantly higher prevalence of both chronic abdominal pain and hospitalization owing to abdominal pain than patients without adhesive SBO. This study had a long duration of follow-up, which is crucial to analyze the true incidence of adhesive SBO and to investigate when it is most likely to occur. Even though most patients developed adhesive SBO during the first 2 years after the initial laparotomy, as reported previously (65,66,145,146), 30% did so after more than 2 years. The mean overall aggregated incidence of adhesive SBO in 11 previous studies was 6.2% (65) compared with 12.6% in the present study. The few previous studies (66–68,146) of adhesive SBO following laparotomy in neonates and infants found a lower incidence of relaparotomy owing to adhesive SBO than the present study. An explanation for this difference might be that the present study group consisted of twofold to threefold more patients with neonatal conditions (such as gastroschisis, malrotation and necrotizing enterocolitis) than in previous reports (65,66,145,146). The inflammatory process in gastroschisis and necrotizing enterocolitis is considered to be a predisposing factor for formation of adhesions (145,147). Approximately 42% of the patients with malrotation had volvulus at surgery, which is also an inflammatory condition. Surgery in patients with malrotation is associated with extensive tissue handling and an increased risk of adhesive SBO (91), which might be minimized by using minimally invasive techniques as reported in adults (35). However, the evidence for less adhesive SBO after laparoscopy in infants is weak. The incidence of adhesive SBO following pyloric stenosis, intussusception and diaphragmatic hernia was similar to rates reported by others (65,66). In contrast to a previous investigation (146), the present study identified four diagnoses – Hirschsprung’s disease, malrotation, intestinal atresia and necrotizing enterocolitis – with a high risk of adhesive SBO requiring relaparotomy. In this study, stoma formation was also identified as a risk factor for adhesive SBO. This observation supports the switch from staged abdominoperineal procedures (148) to transanal endorectal pull-through in Hirschsprung’s disease (not included), as well as the concept of performing, wherever possible, a primary anastomosis rather than creating a stoma in patients with necrotizing enterocolitis or intestinal atresia. In the present study, adhesive SBO after only one previous laparotomy occurred in 54.9% of the patients, slightly lower than the rate reported previously for children (65–76%) (67,149). This study could not confirm that boys had a higher risk of adhesive SBO requiring surgery, as reported previously (149–151). We initially hypothesized that patients who underwent abdominal surgery in 1976–1990 would have a higher incidence of adhesive SBO as a result of infections, inadequate hemostasis and the use of foreign materials (such as catgut and silk sutures, and glove powder) than
patients operated on in the later time interval of 1991–2011. Surprisingly, the incidence of adhesive SBO requiring relaparotomy was similar between the two time periods. One explanation might be that 83% of the patients with necrotizing enterocolitis were operated on during the later time interval, which probably increased the incidence of adhesive SBO. The overall mortality rate in this study was 15.9% (143 of 898 patients), but only three deaths (0.3%) were caused by adhesive SBO, which is similar to rates found in other studies (67,68,149,151). Exclusion of the deceased in this study would increase the mean follow-up time from 14.7 to 17.5 years. The prevalence of self-reported infertility in adult females in the present study was close to the prevalence reported in the general population (76). The strengths of this study are the large number of patients with long follow-up, and that only a small number of patients were lost to follow-up. The main weakness of the study is its retrospective design. The questionnaire was not validated but enabled follow-up of self-reported morbidity, which must be interpreted with caution owing to recall bias.
Conclusions

Early elevation of IL-6, IL-1β and TNF-α concentrations in peritoneal fluid but not in plasma correlate to adhesion formation in our rodent adhesion model. The model enables studies on adhesion formation and the healing process simultaneously. Our data indicate that anti-adhesion strategies should be early, local and not systemic.

PVAC-impregnated sutures might be a new therapeutic approach to be used in combination with adhesion barriers in the reduction of adhesion formation.

The morbidity after laparotomy in neonates and infants is high. Awareness of the risk factors may promote changes in surgical practice.
Future perspectives

Adhesions cause a significant burden of morbidity, mortality and increased economic costs. There are some strategies with anti-adhesion properties but is a reduction in adhesion formation enough to prevent episodes of adhesive small bowel obstruction, chronic abdominal pain and infertility over time? In order to claim true efficacy, future clinical trials need to investigate these complications over time. There is a high incidence of adhesive small bowel obstruction following laparotomy in infants and children have many years to develop complications and would probably benefit the most from an effective anti-adhesion strategy. We will continue our research with impregnated sutures in a larger animal model (pigs or rabbits) with the ambition to bring this adhesion reduction strategy into our clinical practice.
De flesta patienter som genomgått bukkirurgi utvecklar sammanväxningar (adherenser) mellan olika tarmsegment eller mellan tarm och bukvägg. Sammanväxningar kan orsaka kroniska bukmärtor, kvinnlig infertilitet, tarmvred och därmed lidande och ökade sjukvårdskostnader. Tidigare studier på vuxna har visat att mellan 5-20% av tidigare bukopererade patienter måste återinläggas till följd av komplikationer orsakade av sammanväxningar. Barn har en lång förväntad livslängd, varför den sammanlagda risken att utveckla sådana komplikationer borde vara förhöjd jämfört med den hos vuxna men det finns få uppföljningsstudier gjorda på barn som genomgått bukkirurgi. Målen med denna avhandling var dels att etablera en relevant djurmodell med syfte att studera sammanväxningars uppkomst tillsammans med läkningsprocessen, dels att kunna undersöka effekten av potentiella hämmer av sammanväxningar. Ytterligare ett mål var att ta reda på förekomst av och riskfaktorer för operationskrävande tarmvred efter bukkirurgi på spädbarn och att studera patienternas självskattade komplikationer såsom kroniska bukmärtor och infertilitet hos kvinnor.

I delarbete I etablerades en kliniskt relevant, reproducerbar djurmodell där en tidig förhöjning av de inflammatoriska äggviteämnena IL-6, IL-1β och TNF-α i vätska från bukhålan påvisades, och slutsatsen var att hämning av sammanväxningar bör göras tidigt och lokalt i bukhålan.

I delarbete II testades impregnerade suturer (stygns) som impregnerats med PVAC dels för sammanskarvning av två tarmsegment och dels för förslutning av bukväggen. De impregnerade suturerarna minskade förekomsten av sammanväxningar utan att försämra läkningsförmågan (testad genom mätning av den ihopskarvade tarmens bristningstryck).

I delarbete III studerades 898 patienter som bukopererats under sitt första levnadsår. De följes upp under lång tid (mediantid 14,7 år). Etthundratretton av dessa patienter (12,6%) reopereras på grund av tarmvred orsakat av sammanväxningar, 70% av dessa inträffade inom två år efter den första operationen. De fyra sjukdomarna med högst risk för tarmvred var Hirschsprungs sjukdom (risk 29%), malrotation (29%), tunntarmsatresi (28%) och
nekrotiserande enterokolit (25%). Andra riskfaktorer för tarmvred var om operationstiden var längre än en timme, om tillfälliga stomier skapades för att avlasta tarmen och om det inträffade postoperativa komplikationer som sårruptur, läckage i en skarv mellan två tarmsegment eller en varansamling bildades i bukhålan. Av enkäten framkom att sjukhuskrävande vård för bukmärtor var vanligare hos kvinnor (rapporterades hos 32,0%) än hos män (19,2%). Kroniska bukmärtor och sjukhuskrävande vård för bukmärtor var också vanligare i gruppen som opererats för tarmvred efter den första operationen än i gruppen som inte opererats för tarmvred. Ofrivillig barnlöshet rapporterades av 13.8% av kvinnorna över 18 år, vilket inte skiljer sig från normalbefolkningen.
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References


A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)