Modulation of Inflammatory Response in Surgical Trauma

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Thesis defended on September 7th 2012.

Abstract

Surgical trauma results in the activation of inflammatory cascade systems in the body. Excessive systemic inflammation can lead to organ dysfunction in one or several organs. The thesis aims to describe the body's inflammatory response during major surgery and the extent to which the answer depends on different methods of blood salvage, anesthesia and surgical technique.

Method: Twenty-four patients scheduled for total hip arthroplasty were randomized to two groups. Blood was collected via a heparin-coated device or via a non-heparin-coated device. Samples were taken from collected blood to measure quality and inflammatory activation. Fifty consecutive patients who were scheduled for elective open colorectal surgery were included in a prospective and randomized study. The patients were randomized to total intravenous anesthesia with propofol-remifentanil or inhalation anesthesia with sevoflurane. Twenty-four patients with rectal cancer were randomized to open or laparoscopic rectal resection. Blood samples were taken before, during and after surgery for analysis of inflammatory metabolites including cytokines and complement split products.

Results: I: IL-6, IL-8, C3a and SC5b-9 were higher in salvaged blood than in venous blood. There were no significant differences between the blood salvaged in the system with heparin-coated surfaces compared to non-heparin-coated surfaces regarding these parameters. II: IL-6, IL-8 and C3a increased during surgery and were elevated compared to baseline in both groups. III: Bb concentrations increased in both groups during surgery. A significant increase in SC5b-9 concentration was seen in both groups in the postoperative period. IV: IL-6, IL-10 and CRP were higher in the open group as compared to the laparoscopic group.

Conclusions: Blood salvaged intra-operatively during total hip arthroplasty contains elevated levels of complement split products and pro-inflammatory cytokines. Heparin-coated surfaces of the salvage device do not significantly influence the formation of inflammatory mediators. Major colorectal surgery leads to activation of the complement cascade and the release of both pro-inflammatory and anti-inflammatory cytokines. Complement is activated through the alternative pathway. There are no significant differences between total intravenous anesthesia (TIVA) with propofol and remifentanil and inhalational anesthesia with sevoflurane and fentanyl regarding complement activation and the release of pro- and anti-inflammatory interleukins. Rectal surgery causes release of both pro- and anti-inflammatory cytokines. The inflammatory response is lower in laparoscopic rectal surgery as compared to conventional open surgery.

Keywords: inflammatory response, cytokines, complement activation, colorectal surgery, laparoscopy, inhalation anesthesia, intravenous anesthesia, autologous blood transfusion

ISBN 978-91-628-8500-7

Sammanfattning på svenska

Kirurgi leder till aktivering av inflammatoriska kaskadsystem i kroppen. Detta är en normal mekanism för att initiera läkning av kroppsskada. Kraftig systemisk inflammation kan leda till organdysfunktion i ett eller flera organ.

Frågeställning

Denna avhandling syftar till att beskriva kroppens inflammatoriska svar vid stor kirurgi och i vilken utsträckning svaret är beroende av våra olika metoder vad gäller blodåtervinning, narkostyp och kirurgisk teknik. Kan man påverka systemisk inflammation genom val av heparin- eller icke heparinytbehandling på slangar i blodåtergivningssystemet (arbete 1), total intravenös anestesi jämfört med gasanestesi (arbete 2) och laparoskopisk jämfört med öppen stor bukkirurgi (arbete 3 och 4).

Metod

24 patienter som genomgick total höftproteskirurgi randomiserades till 2 grupper. I den ena gruppen användes ett system för autolog blodtransfusion där slangar hade ytbehandlats med heparin. I den andra gruppen saknades denna ytbehandling på slangarna. Prover togs på insamlat blod för att mäta kvalitet och inflammatorisk aktivering.

50 patienter som genomgick öppen kolorektal kirurgi randomiserades till två olika anestesiformer. Den ena gruppen fick total intravenös anestesi med propofol-remifentanil och den andra gruppen fick inhalationsanestesi (Sevoflurane).

Blodprover togs före, under och efter kirurgi för analys av komplementfaktorer och pro- och antiinflammatoriska cytokiner.

24 patienter med rektalcancer randomiserades till öppen eller laparoskopisk kirurgi. Blodprover togs under och efter operation för analys av inflammatoriska metaboliter. I arbete 3 beskrivs komplementaktivering och arbete 4 ligger fokus på pro- och anti-inflammatoriska cytokiner.

Resultat

Det sker en inflammatorisk aktivering med förhöjda nivåer av komplementfaktorer och cytokiner i perioperativt insamlat blod i samband med höftproteskirurgi. Koncentrationen av cytokiner och komplementfaktorer skilde sig inte mellan grupperna.

Under öppen kolorektalkirurgi uppmättes förhöjda nivåer av komplement och cytokiner. Nivåerna skilde sig inte mellan anestesigrupperna.

Förhöjda nivåer av komplementfaktorer uppmättes under och efter laparoskopisk och öppen rektalkirurgi. Komplement aktiveras via den alternativa vägen men ingen skillnad förelåg mellan grupperna.

Under och efter rektalcancerkirurgi uppmättes förhöjda nivåer av CRP, pro- och anti-inflammatoriska cytokiner i båda grupperna. I den laparoskopiska gruppen var nivåerna av IL-6 och CRP signifikant lägre än i den öppna gruppen.

Slutsatser

Insamlat blod under höftproteskirurgi innehåller förhöjda nivåer av komplement och pro-inflammatoriska cytokiner. Heparincoating av slangar påverkar inte blodkvaliteten avseende inflammatoriska metaboliter jämfört med icke heparincoating av slangar.

Avhandlingen visar att det sker en inflammatorisk aktivering under och efter kolorektalkirurgi. Val av narkosform (TIVA eller inhalationsanestesi) påverkar inte nämnvärt nivåerna av inflammatoriska metaboliter.

Laparoskopisk operationsteknik vid kirurgi för rektalcancer ger lägre koncentrationer av vissa inflammatoriska metaboliter jämfört med konventionell öppen kirurgi.

Table of Contents

List of original papers

This thesis is based on the following papers:

Paper I

Kvarnström A, Schmidt A, Tylman M, Jacobsson M, Bengtsson A.

Complement split products and proinflammatory cytokines in intraoperatively salvaged unwashed blood during hip replacement: comparison between heparin-coated and non-heparin-coated autotransfusion systems.

Vox Sang. 2008;95:33-38

Paper II

Kvarnström AL, Sarbinowski RT, Bengtson JP, Jacobsson LM, Bengtsson AL.

Complement activation and interleukin response in major abdominal surgery.

Scand J Immunol. 2012;75:510-516

Paper III

Kvarnström A, Sokolov A, Swartling T, Kurlberg G, Mollnes TE, Bengtsson A.

Alternative pathway activation of complement in laparoscopic and open rectal surgery.

Scand J Immunol. 2012;76:49-53

Paper IV

Kvarnström A, Swartling T, Kurlberg G, Bengtson JP, Bengtsson A.

Pro-inflammatory cytokine release in rectal surgery. Comparison between laparoscopic and open surgical techniques.

Manuscript

Abbreviations

ACTH adrenocorticotropic hormone

ASA American Society of Anesthesiologists

Bb complement factor Bb

C complement

C3 complement factor 3

C3a complement anaphylatoxin 3a

C5a complement anaphylatoxin 5a

C4d complement factor 4d

C9 complement factor 9

CABG coronary artery bypass grafting

cAMP cyclic adenosine monophosphate

CRP c-reactive protein

EDTA ethylene diamine tetraacetic acid

ELISA enzyme-linked immunosorbent assay

E-selectin endothelial-leukocyte adhesion molecule

HLA-DR human leukocyte antigen-DR

IBD inflammatory bowel disease

ICAM-1 intercellular adhesion molecule-1

IL-1 interleukin-1

IL-4 interleukin-4

IL-6 interleukin-6

IL-8 interleukin-8

IL-10 interleukin-10

kD kilodalton

MAC membrane attack complex

MODS multi-organ dysfunction syndrome

MOF multi-organ failure

PCT procalcitonin

PMN polymorphonuclear granulocytes

PVC polyvinyl chloride

S-100 soluble protein in 100% saturated ammonium sulphate

SIRS systemic inflammatory response syndrome

SC5b-9 soluble terminal complement complex

T timepoint

TCC terminal complement complex

TIVA total intravenous anesthesia

TNF-α tumor necrosis factor alpha

VCAM-1 vascular cell adhesion molecule-1

VIMA volatile induction and maintenance of anesthesia

WBC white blood cell count

Introduction

The human body responds to tissue trauma by inflammation. The clinical signs of the inflammatory response are local pain, swelling, and redness. When the tissue is injured the small blood vessels dilate which increases the blood flow to the injured area. The skin turns red and feels warm. Blood-flow is increased locally to make white blood cells and plasma proteins migrate from the circulation to the periphery. The endothelial cells in the vessel wall make space between them to let through white blood cells to the tissue. We notice this as swelling of the injured body part. The protein-rich fluid that leaks from the circulation is also known as exudate.[1] The white blood cells and plasma proteins are necessary to minimize harm and to induce the healing process. Sir David Cuthbertson first described the normal inflammatory response to surgery. He divided the stress response in two phases. The initial "ebb" phase is characterized by peripheral vasoconstriction and redistribution of blood to central organs.[2] Energy expenditure is generally low and conservation of salt and fluid is increased. Today we know this initial phase as the shock phase.[3]

After resuscitation of the circulating volume and survival of the shock phase, the second phase, which Cuthbertson named "flow" phase starts.[2] The flow phase is typically hypermetabolic with increase in energy expenditure and oxygen consumption. Catabolism of muscle protein creates a substrate for gluconeogenesis in the liver.[4] Cardiac output is increased and the peripheral blood vessels are dilated to ensure delivery of oxygen and glucose to the tissue. Minute ventilation is increased due to increased demand of O_2 and for excretion of CO_2. The systemic inflammatory response syndrome, SIRS, describes a state of prolonged hypermetabolism.[5] Markers of the inflammatory system are elevated after surgical trauma. C-reactive protein (CRP), interleukins and complement split products are examples of markers of the systemic inflammatory response.[6] Extensive systemic

inflammation can lead to depressed immunity and susceptibility to infections.[7] Multi-organ dysfunction syndrome (MODS) and later multi-organ failure (MOF) can develop and eventually be fatal.[8] The degree of immunomodulation after surgery is dependent on a variety of factors. The extent of tissue trauma, type of anesthesia, co-existing diseases, blood transfusions and the use of extra-corporeal circulation are all factors that can modulate the inflammatory response.

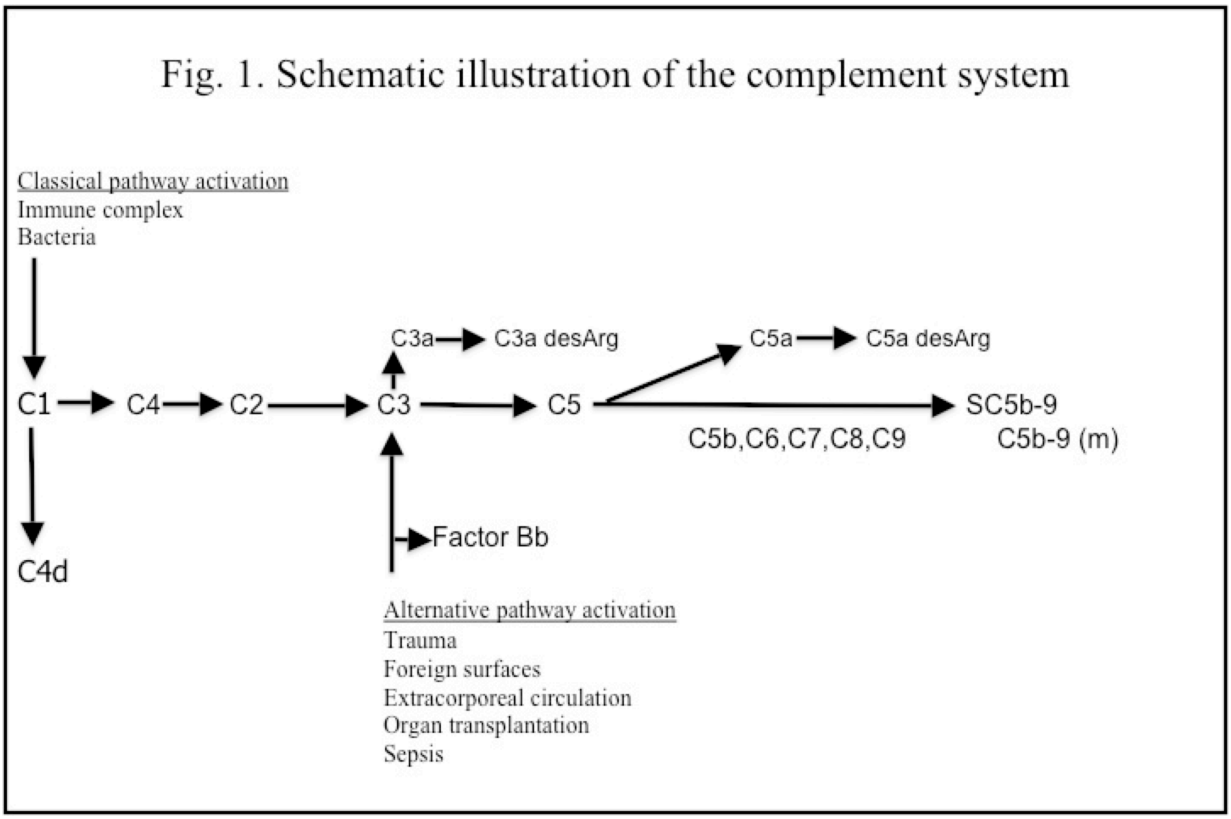
The complement system

The complement system is a group of proteins that have important effects in the defense against bacteria.[9] It includes more than 30 different proteins found in blood plasma and on cell surfaces. The proteins of the complement cascade are mainly produced in the liver. The proteins of the classical pathway are numbered from C1 to C9. The classical pathway is triggered by the interaction between antigen and antibodies.[10] The alternative pathway of complement activation was discovered later. The proteins of the alternative pathway are called factors followed by a letter, for example factor D. The alternative pathway starts at C3. C3b-fragments on bacterial surfaces forms a C3-convertase together with factor B. The alternative pathway is a reinforcement to the classical pathway and speeds up the opsonization process. There is also a third activation pathway; the mannose-binding pathway. Complement is activated by bacteria but also by tissue trauma and blood contact with foreign surfaces.[11,12] Complement is important in the host defense against infections. This is due to the opsonization effect of complement.[13] Bacteria dressed with complement 3b (C3b) on the surface are more easily fagocytosed than bacteria without. The C3b molecules interact with receptors on the surface of granulocytes, monocytes and macrophages. The target cell adheres to the granulocyte or the macrophage and is then fagocytosed.[14] Complement also has a lytic ability and can cause destruction and lysis of the target cell. The final step of complement activation is formation of the membrane attack complex (MAC or SC5b-9). The

membrane attack complex is initiated when C5 is cleaved. The proteins C5-C9 together form a channel through the surface membrane of the target cell.

When complement is activated, small proteins, anaphylatoxins, are released.[15] The anaphylatoxins are small proteins with effects on the blood vessel wall. C3a and C5a are the most potent anaphylatoxins and they cause vasodilatation and increased vascular permeability.[16] The anaphylatoxin C5a also works as a chemotactic protein. When C5a is released this attracts neutrophilic granulocytes to the site of complement activation. Systemic complement activation generates anaphylatoxins within the vessels and that causes granulocytes to aggregate and form emboli in the pulmonary circulation.[17] This might be a contributing factor to development of ARDS (acute respiratory distress syndrome) in trauma patients.

Fig. 1. Schematic illustration of the complement system



Cytokines

Cytokines are produced as part of the inflammatory response to tissue trauma and infection. Many of the symptoms we experience during an infection are the systemic effects of cytokines. Fever, fatigue, increased production of cortisol and acute phase proteins are symptoms that are initiated by cytokines. IL-1, IL-2 and IL-6 are responsible for causing fever during systemic inflammation.

Cytokines, or interleukins, are messengers of the immune system.[18] They are small proteins with a size of 8-25 kD. Cytokines are signal molecules that recruit inflammatory cells to the tissue from the circulation and activate them.[19] These signals have an effect on metabolism, hormones and brain function. Cytokines are not stored in inflammatory cells. When cells receive an inflammatory signal synthetization of cytokines starts within hours. Cytokines bind to a specific receptor at the surface of the target cell. They often affect cells in the immediate vicinity, so called paracrine signalling.[1] Some cytokines such as IL-6 work as endocrine signals and act on target cells in other parts of the body.[20] There are interleukins with pro-inflammatory effects and there are cytokines with anti-inflammatory abilities.

Interleukin-1 and **TNF-α** are cytokines with typical pro-inflammatory characteristics.[21] Alarm signals such as presence of bacteria or dying cells initiates production of IL-1 and TNF-α primarily by macrophages. Both IL-1 and TNF-α promote recruitment of neutrophils from the circulation to the site of inflammation. IL-1 induces fever, loss of appetite, muscle ache, and in higher concentrations, hypotension and multi-organ dysfunction.[22] TNF-α is mainly produced by macrophages but also by T-cells and mast cells. The effects and symptoms of TNF-α are the same as the effects of IL-1.[23]

IL-6 is a cytokine with a variety of effects on many different cell types. Interleukin-6 induces production of antibodies by B-cells.[24] Mediation of the acute phase response is another important function. The increased production of acute

phase proteins following an inflammatory stimulus includes clotting factors, complement and transport proteins.[25] Monocytes and macrophages produce IL-6 but IL-6 is also produced by endothelial cells, epithelial cells, T-cells and B-cells.[20] The acute phase response is often considered a pro-inflammatory response but there are also anti-inflammatory effects that limit the tissue injury.[26] C-reactive protein (CRP) promotes phagocytosis of bacteria by activation of complement. Bacterial surfaces are then dressed with complement proteins and are therefore more easily phagocytosed.[27] Interleukin-6 stimulates platelet production and recruitment of neutrophils to the site of inflammation.[20] Many of the effects of IL-6 are anti-inflammatory and it is incorrect to classify IL-6 as strictly pro-inflammatory. The levels of IL-6 are high during infection and inflammation but that is due to stimulation by the pure pro-inflammatory cytokines Il-1 and TNF.[28] In vitro IL-6 actually inhibits production of TNF-α. IL-6 also has effects in the neuroendocrine system. For example IL-6 induces release of adrenocorticotrophic hormone (ACTH) from the pituitary gland which in turn increases release of cortisol from the adrenal glands.[29]

IL-8 and other chemochines are cytokines that attracts cells from the circulation and recruit them to the site of inflammation.[30] They are relatively small cytokines (6-14 kD), some of them are active in the normal circulation of lymphocytes from the systemic circulation to the lymphatic nodes. Others are only expressed during inflammation to recruit specific leukocytes to the site of inflammation. One example is IL-8 which is produced by monocytes and macrophages and can also be produced by endothelial cells which have been stimulated by IL-1 and TNF-α. The neutrophil granulocyte interacts with IL-8 which is expressed on the vessel wall and gets ready to leave the circulation. When the neutrophil is in the tissue it moves towards the gradient of IL-8. [1]

IL-10 is a cytokine with anti-inflammatory function and immuno-regulatory function.[31] It is produced by both monocytes, macrophages and activated T-cells.

IL-10 stimulates production of antibodies, inhibits antigen presentation and production of pro-inflammatory cytokines.[1,32]

Autologous blood transfusion

Autologous blood transfusion is the collection and transfusion of the patient's own blood. The main purpose of autologous transfusion is to eliminate the risk of incompatibility and blood-borne diseases caused by allogeneic transfusion. Evidence from clinical trials shows that autologous transfusion is more cost-effective than allogenic transfusions and that postoperative infections are reduced.[33-35]

Transfusion of autologous blood is applied primarily in elective surgery。There are a number of different principles for autologous transfusion, including pre-deposit transfusion, intra-operative hemodilution, and intra-operative and postoperative salvage.[36] Intra-operative cell salvage can be described as aspiration and retransfusion of blood from the operative field. The collected blood is centrifuged and washed by the cell-saving device before retransfusion. Anticoagulant is added, and the blood passes through a filter to remove debris.

Salvaged blood that is not centrifuged and washed contains elevated concentrations of various inflammatory mediators. It has been shown that such blood contains high concentrations of complement split products and pro-inflammatory cytokines that may contribute to morbidity after surgery.[37,38] This has led clinicians to limit the amount of filtered blood transfusion to a maximum of 1.5 liters. Transfusion of postoperatively salvaged and filtered blood within this range is considered safe.[39,40] The anticoagulant heparin helps to prevent the formation of blood clots and somewhat improved biocompatibility in terms of leukocyte activation, complement activation and pro-inflammatory cytokine release. The heparin-coated surfaces have been shown to reduce complement activation and cytokine release in extra-

corporeal circulation.[41,42] Our study was conducted to investigate the quality of the intra-operatively salvaged blood regarding levels of cytokines and complement split products. We were also interested in whether heparin-coating of the tubing in the salvage device, would decrease levels of inflammatory mediators in salvaged blood.

Anesthesia and inflammation

The surgical trauma triggers a systemic stress response. Surgery can be performed under general or regional anesthesia. The type of anesthesia chosen for the procedure also affects the systemic stress response. The use of epidural blockade during major abdominal surgery attenuates the stress response to surgery and prevents impairment of lymphocyte function.[43] Opioids suppress secretion of hormones from hypothalamus and the pituitary gland. At the hypothalamic level the release of ACTH is blocked and, as a consequence, also release of cortisol from the adrenal glands.[44] Crozier and colleagues found that propofol-alfentanil anesthesia causes a decreased pro-inflammatory response with lower levels of IL-6 as compared with patients anesthetized with isoflurane . They suggested that this was an alfentanil mediated effect on opioid receptors which leads to reduced intracellular cyclic adenosine monophosphate (cAMP). This second messenger mediates release of IL-6.[45] In a study performed by Schneemilch et al patients subject to partial discectomy were randomized to total intravenous anesthesia with propofol and alfentanil or inhalational anesthesia with sevoflurane.[46] The levels of IL-6 were higher in the group receiving sevoflurane compared to propofol-alfentanil. The expression of HLA-DR molecules on leukocytes was more suppressed in the sevoflurane-group, suggesting a more pronounced inflammatory response to surgery under balanced inhalational anesthesia. We hypothesized that total intravenous anesthesia with propofol and remifentanil leads to lower systemic levels of complement split products and interleukins compared with inhalational anesthesia with sevoflurane and fentanyl.

Minimally invasive surgery and inflammation

Surgical trauma induces a temporary period of immunosuppression that may increase the susceptibility to postoperative infection.[47] By using minimally invasive surgical techniques we can affect the level of systemic inflammation. Several studies in different kinds of patients show a benefit for the patient in terms of earlier mobilization, more rapid return to normal activity and less pain.[48,49] Biochemical investigations also show a lower degree of systemic inflammation during laparoscopic as compared to open surgery.[50] To preserve the patients immune system is important to prevent postoperative infections and to prevent port-site metastasis of tumor cells. Laparoscopic surgery seems to induce a smaller tissue injury as compared to open surgery and thereby a decreased inflammatory response.[51] The faster recovery after laparoscopic surgery may be explained by the immunologic advantage. The smaller skin incisions and less manipulation of bowel and organs contribute to this immunologic advantage. In laparoscopic surgery the establishment of a CO_2 pneumoperitoneum minimizes the exposure to room air. Exposure of the abdominal cavity to room air increases the local and systemic inflammatory response compared to CO_2.[52-54] Both conventional laparotomy and laparoscopy provoke an increase of pro-inflammatory cytokines in the circulation.[55] Increased levels of IL-6 can be measured within hours after start of surgery.[56] High plasma concentrations of IL-6 are associated with development of multi-organ failure and mortality in trauma patients and also after abdominal aortic aneurysm surgery.[57-59] Several studies, in different types of surgery, have shown lower levels of IL-6 in laparoscopic surgery than conventional open surgery. Cholecystectomy and colectomy are examples where lower plasma concentrations of IL-6 have been measured in laparoscopic surgery.[56,60] The exceptions are smaller surgery with less extensive tissue trauma, for example inguinal hernia repair, where laparoscopic surgery does not show an immunologic advantage.[61] Regarding C-reactive protein (CRP) the pattern is similar to IL-6. Studies on laparoscopic and open cholecystectomy show significantly lower levels of CRP after laparoscopic surgery

compared to conventional open surgery.[62] Also the release of the pro-inflammatory cytokine IL-1 has been reported as lower in laparoscopic cholecystectomy as compared to open.[63] In summary, most research on the subject laparoscopic versus abdominal surgery and inflammatory response has been done on cholecystectomy and relatively minor surgery.

The function of the cell-mediated immunologic response is also affected by surgery. Stress caused by surgical trauma impairs the function of polymorphonuclear and mononuclear cells.[64] That may lead to postoperative wound infections and sepsis. The expression of class II major histocompatibility complex (HLA-DR) on the surface of leukocytes is important for antigen presentation and the specific immune response. A low expression of HLA-DR has been associated with infectious complications after surgery.[65-67] Previous studies on laparoscopic cholecystectomy have shown that the expression of HLA-DR is preserved during surgery. In open cholecystectomy the expression of HLA-DR on monocytes was decreased 1 day after surgery.[68,69] Surgery also affects the function of T-cells. Laparoscopic cholecystectomy is associated with a reduced alteration in the ratio of T-helper to T-suppressor cells.[64] In animal models, peritoneal macrophages are less activated by laparoscopy compared to laparotomy.[70,71] Minimally invasive surgery induces a smaller injury than conventional laparotomy and thereby proportionally decreased immunologic changes.

Aims and objectives

The aims of this thesis were to:

1. Investigate and measure the degree of inflammatory response in intraoperatively salvaged autologous blood during hip replacement surgery.

2. Examine whether heparinization of the PVC-tubing in the blood collection device affects the inflammatory response.

3. Investigate and measure the degree of systemic inflammatory response during major colorectal surgery.

4. Determine whether the type of anesthesia affects the inflammatory response.

5. Determine whether the surgical technique affects the inflammatory response.

Methods

Patients

All studies were approved by the Regional Ethical Review Board of Gothenburg and conducted in accordance with the principles stated in the Declaration of Helsinki. Written informed consent was obtained from all participating patients.

Study I

Twenty-four consecutive patients scheduled for total hip arthroplasty. The patient was subjected to a total hip arthroplasty and was over 18 years of age. The patients were randomized to one of two groups. In Group 1 (n=12) the blood was collected via a heparin-coated device and in Group 2 (n=12) the blood was collected via a non-heparin-coated device. There were no significant differences between the groups regarding age.

Study II

Fifty consecutive patients who were scheduled for elective open colorectal surgery were included in this prospective randomized study. Thirty-two male and eighteen female patients were included in the study. The patients underwent open colorectal surgery such as anterior rectal resection, colectomy or rectal amputation. In forty-four patients the indication for operation was rectal cancer, and four patients were operated on due to inflammatory bowel disease, Crohns disease or ulcerative colitis. Two patients had both inflammatory bowel disease and colo-rectal cancer.

Studies III and IV

Patients subject to rectal surgery due to rectal cancer were included in the study. The study was prospective and randomized. Twenty-four patients were included in the study, 13 male and 11 female. The patients were randomized to either laparoscopic surgery (n=12) or conventional open surgery (n=12). The patients were subject to anterior rectal resection or abdomino-perineal resection due to cancer.

Statistical methods

Study I

The sample size was considered in the design of the study. An estimation of a total of 24 patients (12 in each group) was deemed sufficient to detect differences in collected blood. Statistical evaluation: Descriptive statistics were calculated for all variables. The Wilcoxon Rank Sum test (exact), two-sided, was used for all comparisons between the two groups. The Wilcoxon signed rank test was used for comparisons within each group with regard to intra-individual change over time.

Study II

Differences in age, duration of anesthesia and surgery, blood-loss, American Society of Anesthesiologists (ASA) physical status classification scores and length of stay at hospital postoperatively were tested between the treatment groups using Mann-Whitney U tests. Chi-squared tests were used to compare proportions. Mean values of each inflammatory marker for each anesthetic treatment group at each measurement point were plotted and inspected visually. These repeated measurements were then analyzed using linear mixed models with an unstructured

covariance structure and maximum likelihood estimates for both all patients and those without inflammatory bowel disease (IBD).

Study III

The repeated measures of continuous data from each patient were analyzed using linear mixed models with type of surgery, time and the interaction term as fixed factors.

Study IV

The repeated measures of continuous data from each patient were analyzed using linear mixed models with type of surgery, time and the interaction term as fixed factors. Statistical significance was determined with p-values less than .05. To estimate the sample size required a power analysis was performed. The hypothesis was based on an expected 30% lower activation rate of IL-6 and IL-8 in the laparoscopic group compared to the open surgical group. This was based on a significance criterion of .05.

All exploratory and formal statistical tests were carried out using SPSS for Windows (Version 18, SPSS Inc, Chicago, Il, USA), all tests were two-tailed and statistical significance was determined with p-values less than 0.05 (Studies II, III, IV).

Blood sampling and laboratory method

Study I

Blood samples were drawn before the operation and after induction of anesthesia. The collection system was connected to the surgical suction device and the initial 200 ml of shed blood was collected and transferred to the blood bag from which blood samples were taken. Pro-inflammatory cytokines, IL-1β, IL-6, IL-8 and TNF-α were determined with a commercially available EIA system (Endogen, Pierce, IL, USA). C3a and SC5b-9 were measured with a Quidel enzyme-linked immunosorbent assay (San Diego, CA, USA). PMN-elastase, determined as the granulocyte- α 1-protease inhibitor complex, was analyzed by an enzyme linked immunosorbent assay (Milenia, Biotek, GMBH, Germany). Blood samples for complement and cytokine determinations were drawn into tubes for plasma, containing ethylene diamine tetraacetic acid (EDTA) added in the proportion of 0.34 M EDTA per 4.5 ml of blood. The tubes were centrifuged to remove cells, and the samples were frozen within 30 minutes in individual tubes for different determinations and stored at –80^{o}C until all measurements were completed. All assays were analyzed in duplicate. Hb (hemoglobin), Hct (hematocrit), WBC (white cells), PLT (platelets), p-Hb (plasma hemoglobin), sodium, potassium and pD-Dimer were measured by standard methods used for clinical purposes at the Department of Clinical Chemistry, Sahlgrenska University Hospital, Gothenburg, Sweden.

Study II

Blood samples were drawn at four times before, during and after surgery. The first sample (T0) was drawn after insertion of the arterial line before induction of anesthesia. The subsequent samples T1, T2 and T3 were taken at 60 minutes after start of surgery, 30 minutes after completion of surgery and 24 hours after the end

of surgery respectively. Blood-samples were collected in tubes coated with ethylene diamine tetraacetic acid (EDTA); 7.2 mg EDTA per 4.5 ml blood. After centrifugation for removal of cells the samples were frozen within 30 minutes and stored at – 80°C. The blood samples provided data on the levels of complement split products (C3a and SC5b-9), pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) and anti-inflammatory cytokines (IL-4 and IL-10).

The levels of TNF-α, IL-1β, IL-4, IL-6, IL-8 and IL-10 were obtained with SearchLight method (Pierce Biotechnology, MA, USA) which is a multiplex sandwich enzyme-linked immunosorbent assay (ELISA) in a planar, plate-based array format, for the quantitative measurement of secreted proteins in different biological materials. Diluted samples and controls were incubated for one hour on the arrayed plates. All incubations were performed at room temperature with shaking at 200 rpm. The plates were decanted and washed six times before adding a cocktail of biotinylated detection antibodies to each well. After incubating with detection antibodies for 30 minutes, the plates were washed three times and incubated for 30 minutes with streptavidin-horseradish peroxidase. The plates were again washed before adding SuperSignal Femto Chemiluminescent substrate. The plates were immediately imaged using the SearchLight Black Ice imaging system, and data was analyzed using Array Analyst software (Auchon Biosystems, Billerica, MA, USA). All results were in the range of the standard curve.

Studies III and IV

Blood samples for determination of complement activation (C4d, C3bc, Bb and the terminal C5b-9 complex TCC) were drawn during surgery and in the postoperative period. Samples were taken at five separate time points. The first sample was taken after induction of anesthesia, before start of surgery (T0). The next samples were taken at the following time-points after start of surgery: 180 minutes (T1), 360 minutes (T2), 24 hours (T3) and late in the postoperative period (3-5 days after

surgery depending on which day the patient was operated on) (T4). Arterial blood-samples were collected in tubes coated with ethylene diamine tetraacetic acid (EDTA); 0.34 M EDTA per 4.5 ml blood. The last blood-sample (T4) was a venous sample since the arterial line was removed when the patients left the postoperative recovery unit. After centrifugation plasma was separated, frozen within 30 minutes and stored at – 80°C for later enzyme-linked immunosorbent assays (ELISA) analysis of complement activation products.

C4d and Bb were measured using ELISA according to the instructions from the manufacturer (Quidel, San Diego, CA, USA). The C3bc concentration was measured by an ELISA based on the mouse anti-human C3bc antibody (clone bH6).[72] The assay has been described in detail previously. The TCC concentration was measured by an ELISA based on the mouse anti-human TCC antibody (clone aE11) reacting with a neoepitope exposed in C9 when incorporated into C5b-9. The assay has been described in detail previously[73], and was performed according to a later modification.[74]

The levels of IL-1α, IL-6, IL-8, IL-10, TNF-α, ICAM-1 and VCAM-1 were obtained with SearchLight methods (Pierce Biotechnology, MA, USA) which is a multiplex sandwich enzyme-linked immunosorbent assay (ELISA). Described in detail on the previous page under study II. The levels of CRP and WBC were measured at the hospital laboratory for clinical chemistry.

Anesthesia method

Study I

Spinal anesthesia with 15-20 mg bupivacaine (Marcain®, AstraZeneca, Södertälje, Sweden) and 0.2 mg morphine hydrochloride (Morfin Special, AstraZeneca, Södertälje, Sweden) was used for surgery.

Study II

Both groups: Before induction 1 mg of midazolam (Dormicum®, Roche AB, Stockholm, Sweden) was given intravenously and an arterial line was inserted in the left radial artery for repeated blood analyses and continuous blood pressure monitoring. A thoracic epidural catheter was inserted in the thoracic VII-XII interval. All patients also received 0.5 mg of atropine (Atropin Merck NM, Merck NM AB, Stockholm, Sweden) before induction of anesthesia. Before endotracheal intubation fentanyl (Leptanal®, Janssen-Cilag AB, Sollentuna, Sweden) and rocuronium (Esmeron®, Organon AB, Göteborg, Sweden) were given in standard doses. A continuous epidural infusion was started during the operation with bupivacain 5 mg/ml (Marcain® adrenalin, AstraZeneca AB, Södertälje, Sweden) and epinephrine 5 μg/ml at an infusion rate of 4-6 ml/h. At the end of the operation, the patients were given 5-10 mg of ketobemidon (Ketogan®, Pfizer AB, Sollentuna, Sweden) which is equipotent to 7-15 mg of morphine.

Group TIVA: Patients were anesthetized with total intravenous technique; a combination of propofol (Diprivan®, AstraZeneca AB, Södertälje, Sweden) and remifentanil (Ultiva®, Glaxo Smith Kline AB, Solna, Sweden) was used. Propofol was administered intravenously with Target Controlled Infusion (Alaris Diprifusor® IVAC TCI and TIVA, Alaris Medical Systems Ltd, Hampshire, UK). The target concentration during induction was 3 μg/ml. The target concentration was decreased to 2 μg/ml during the operation. Remifentanil was administered as a continuous intravenous infusion. The infusion rate at induction was 0.25 μg kg^{-1} min^{-1}. The infusion rate was then lowered to 0.15 μg kg^{-1} min^{-1} during surgery.

Group INHALATION: The patients received inhalation anesthesia with sevoflurane/O_2/air. Sevoflurane was used both as induction agent and for maintenance of anesthesia (VIMA, Volatile Induction and Maintenance of Anesthesia). Anesthesia was induced by inhalation of a mixture of sevoflurane/O_2/air (Sevorane®, Abbott Scandinavia AB, Solna, Sweden). For

maintenance, the end-tidal sevoflurane concentration was kept at 1.4-2.8 vol%. Fentanyl, in repeated intravenous doses of 25-100 μg, was given at the discretion of the anesthesiologist.

Studies III and IV

All patients were under general inhalation anesthesia. Anesthesia was induced by administration of propofol (Diprivan®, AstraZeneca AB, Södertälje, Sweden) intravenously. Before endotracheal intubation the patients were given fentanyl and rocuronium (Leptanal®, Janssen-Cilag AB, Sollentuna, Sweden and Esmeron®, Organon AB, Göteborg Sweden).

Anesthesia was maintained by inhalation of a mixture of sevoflurane/N_2O/O_2 (Sevorane®, Abbot Scandinavia AB, Solna, Sweden). After induction of anesthesia an arterial line was placed in the left radial artery for continuous monitoring of blood pressure and repeated blood samplings. For postoperative analgesia the patients received an epidural catheter before induction of anesthesia. The epidural was activated at the end of the operation.

Description of the intra-operative blood collection system (Study I)

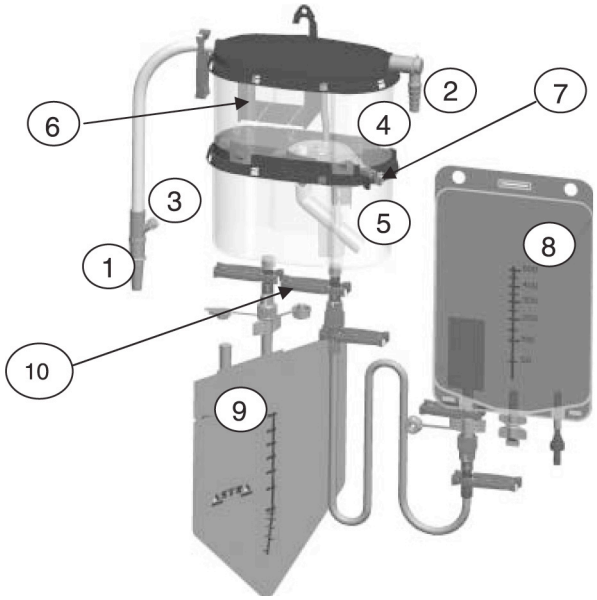
A new intra-operative autologous blood transfusion system was evaluated (Sangvia®, AstraTech, Mölndal, Sweden). The system contains a suction unit that is attached to the inlet and a vacuum connector connected to a vacuum source. Citrate is added to the collected blood through a citrate port. Blood is aspirated with a suction unit, filtered in the 200-micron blood filter before it is collected via an upper canister in a lower canister. When a sufficient amount of blood has been collected in the lower canister, a slide clamp between the lower canister and a blood bag is opened, the connection between the canister is closed and blood can be transported to the blood bag. Blood is meanwhile collected in the upper canister.

After the blood is transported to the blood bag, the outlet slide clamps are closed, the slider is opened and blood is again collected in the lower canister. The system is schematically illustrated in figure 2.

Heparin coating

The suction unit tubing and the inlet tube were coated with heparin using Carmeda CBAS technology. The heparin was slightly modified, resulting in a reactive aldehyde group at one end of the molecule. The aldehyde group is covalently bond to an amino group on the material surface (tubing surface) and gives a stable non leaching heparin coating, compared to non covalently bond coating techniques. The functionality of the heparin molecule is also preserved using a covalent bond, since the functional groups of the molecule are free to react.

Fig. 2. Intra-operative auto-transfusion system (Sangvia®, AstraTech, Sweden).



(1) Connection for suction unit, (2) vacuum connector, (3) citrate port, (4) upper canister (5) lower canister, (6) 200 micron filter, (7) slider, (8) blood bag, (9) waste bag and (10) slide clamp.

Results

Study 1

Compared to venous blood, Hb, Hct and WBC were significantly decreased in salvaged blood ($p<0.05$, in both groups). Plasma Hb, sodium and potassium were higher in salvaged blood than in venous blood ($p<0.001$, in both groups). There were no significant differences in Hb, Hct, WBC, PLT, p-Hb, sodium, potassium, pD-dimer and base excess between the blood salvaged in the system with heparin-coated surfaces compared to non- heparin-coated surfaces.

The pro-inflammatory cytokines IL-6 and IL-8 were higher in salvaged blood than in venous blood ($p<0.05$, in both groups). IL-1β and TNF-α were not significantly altered in collected blood. There were no significant differences regarding pro-inflammatory cytokines between blood salvaged in the system with heparin-coated surfaces compared to non- heparin-coated surfaces. Complement anaphylatoxin C3a increased significantly in salvaged blood ($p<0.001$, in both groups) while SC5b-9 was only significantly elevated in group 1 ($p<0.05$), the heparin-coated system. There were no significant differences regarding C3a and SC5b-9 between the blood salvaged in the system with heparin-coated surfaces compared to non-heparin-coated surfaces. The plasma concentration of PMN-elastase was elevated in salvaged blood compared to venous blood ($p<0.001$, in both groups).

Study 2

The C3a levels were increased during surgery in both groups compared to baseline ($p<0.001$). The concentration of C3a had a biphasic course in both groups; decreasing to preoperative values at T2 (30 minutes after surgery) only to rise again during the next 24 hours. A decrease in the levels of SC5b-9 compared to pre-operative values was seen in both groups during surgery ($p<0.001$). No significant

differences regarding levels of C3a and SC5b-9 were recorded between the treatment groups.

The levels of the pro-inflammatory cytokines IL-6 and IL-8 increased during surgery and were elevated ($p<0.001$) compared to baseline. No significant differences between the two groups were recorded for either cytokine. IL-6 reached a peak median concentration at T2 (30 minutes after surgery). There were no significant differences between groups regarding concentrations of IL-6 at any time. The pro-inflammatory cytokine IL-8 followed a similar pattern over time. No significant differences were recorded between the two groups. Regarding TNF-α and IL-1β there was not an elevated concentration in any of the studied groups at any occasion.

The concentration of the anti-inflammatory cytokine IL-10 was elevated in both groups. There was a significant change in concentration of IL-10 compared to baseline in both groups ($p<0.001$) over time but no difference between the treatment groups. Regarding the concentration of IL-4 there was no significant difference in concentration over time or any difference between the treatment groups.

Linear mixed models did not identify any significant interactions between time and anesthetic type nor any significant pairwise comparisons at each time point after baseline. The analyses performed excluding patients with IBD (inflammatory bowel disease) again showed no significant differences between anesthetic groups.

Fig. 3. Mean changes in IL-6 from pre-op baseline to 24 h post-op by type of anesthetic.

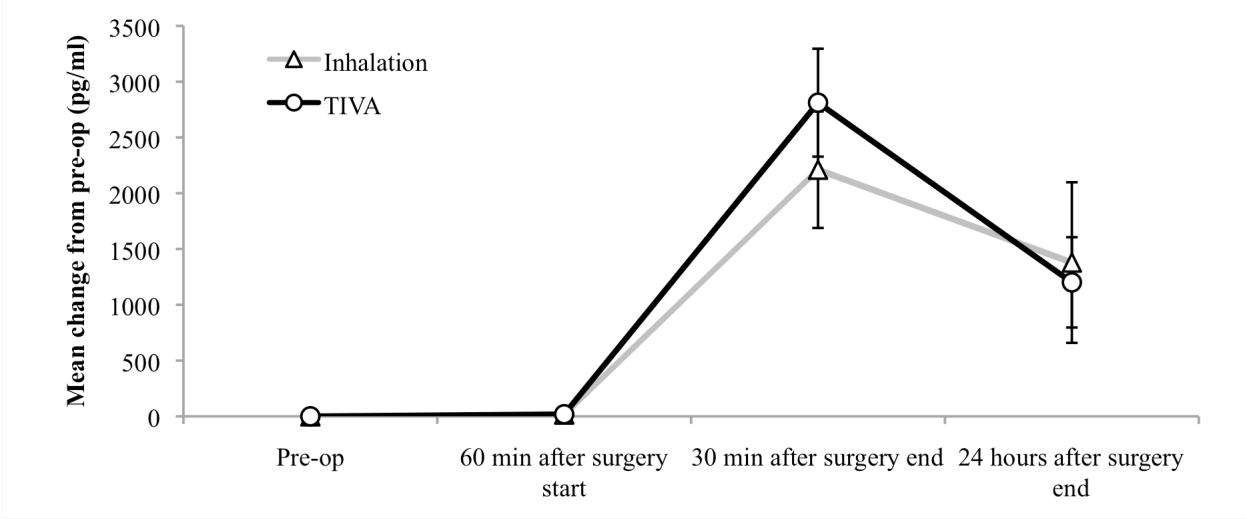


Fig. 4. Mean changes in IL-8 from pre-op baseline to 24 h post-op by type of anesthetic.

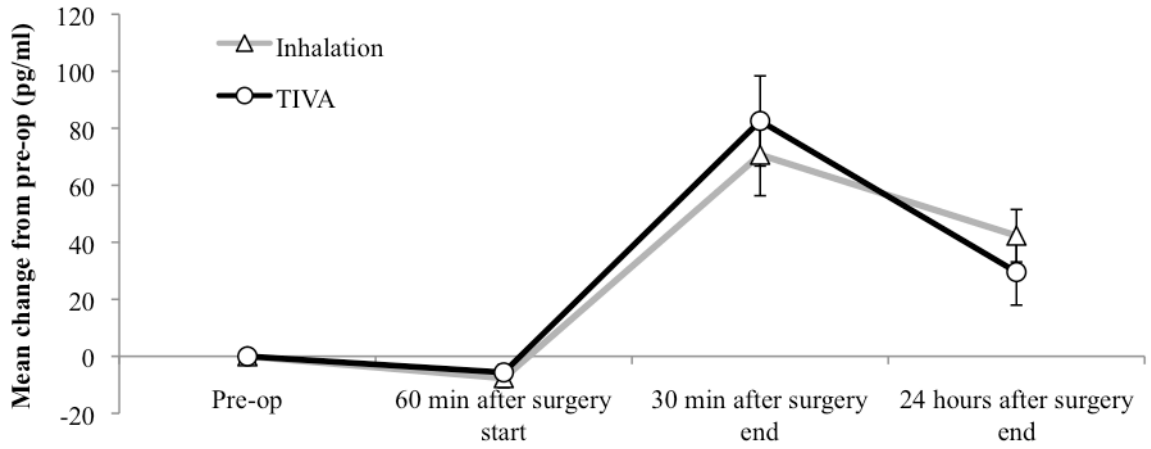


Fig. 5. Mean changes in IL-10 from pre-op baseline to 24 h post-op by type of anesthetic.

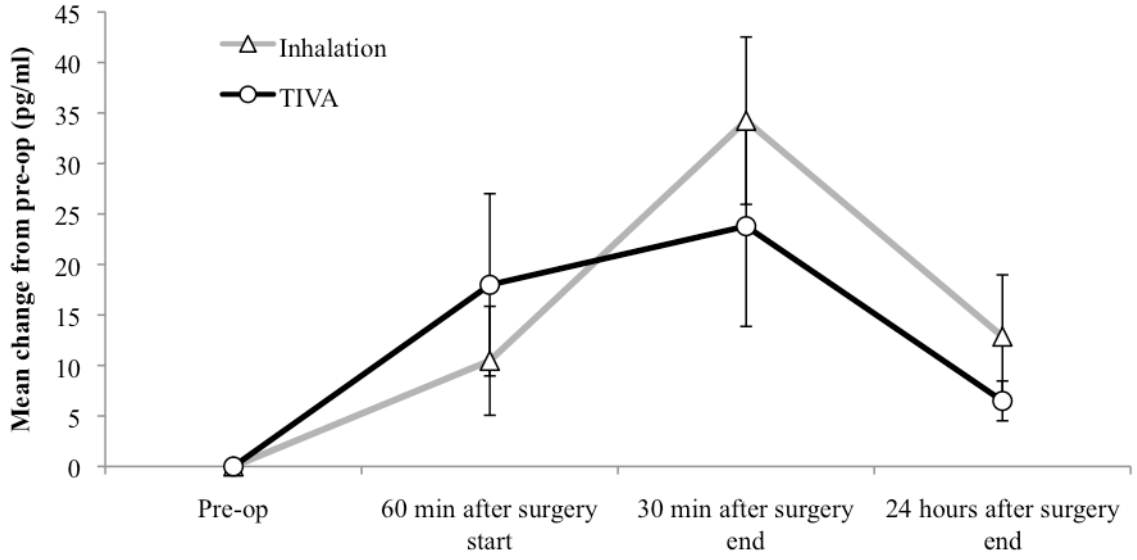


Fig. 6. Mean changes in C3a from pre-op baseline to 24 h post-op by type of anesthetic.

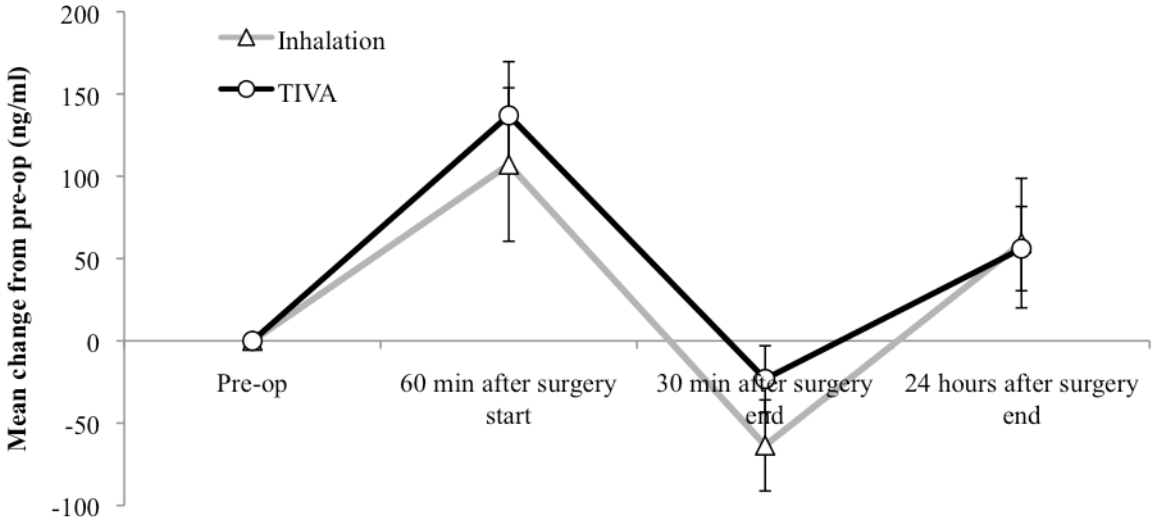


Fig. 7. Mean changes in SC5b-9 from pre-op baseline to 24 h post-op by anesthetic type.

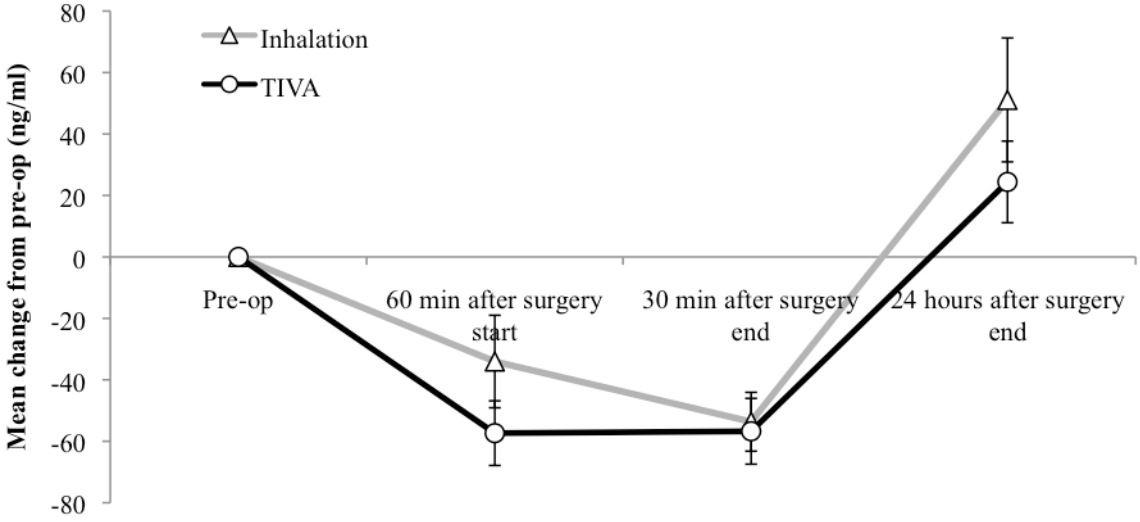
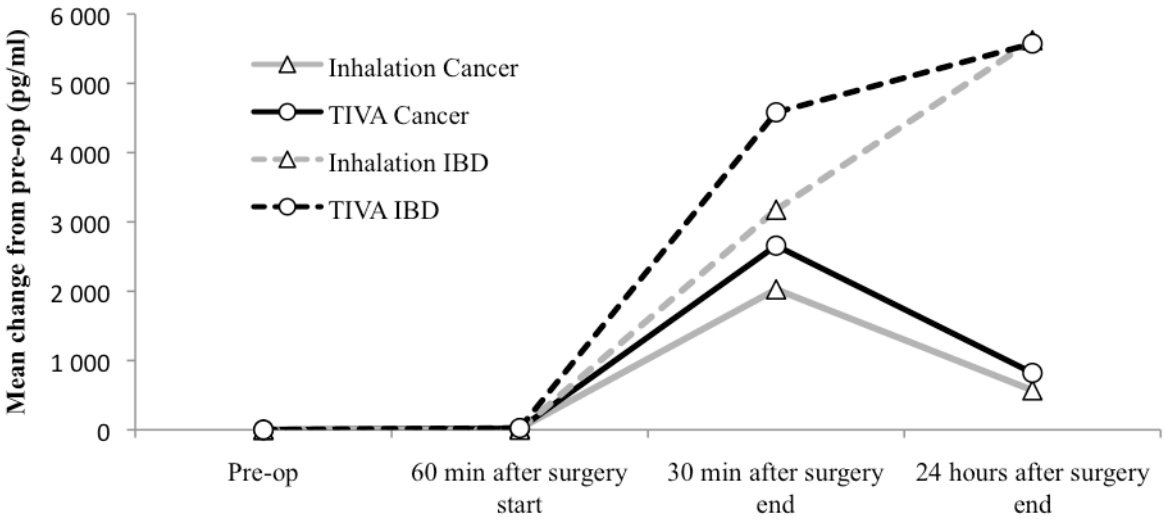


Fig. 8. Mean changes in IL-6 from pre-op baseline to 24 h post-op by anesthetic type for patients with IBD and colorectal cancer.



Study 3

Bb: Bb concentrations increased significantly ($p<0.001$) in both groups during surgery. The concentrations then declined in both groups and reached baseline level at T4 (3-5 days postoperatively). The concentrations of Bb were significantly lower in Group 1 (laparoscopic) at T1 ($p=0.043$) compared to Group 2 (open).

TCC: A significant increase in TCC concentration was seen in both groups in the postoperative period. Peak concentrations were measured in the late postoperative period 3-5 days after surgery (T4). There were no significant differences between the groups.

C3bc: The concentration decreased in both groups at T1 and T2 compared to baseline level. The change over time was significant in both groups but there was no significant difference between the groups.

C4d: C4d showed a similar pattern as C3bc with a "U-shaped" curve, decreasing at T1 in both groups compared to baseline concentrations. C4d gradually returned to baseline levels. The change over time was significant in both groups but there was not a significant difference between groups.

Fig. 9. Mean plasma concentration of factor Bb by type of surgery and hours after pre-op.

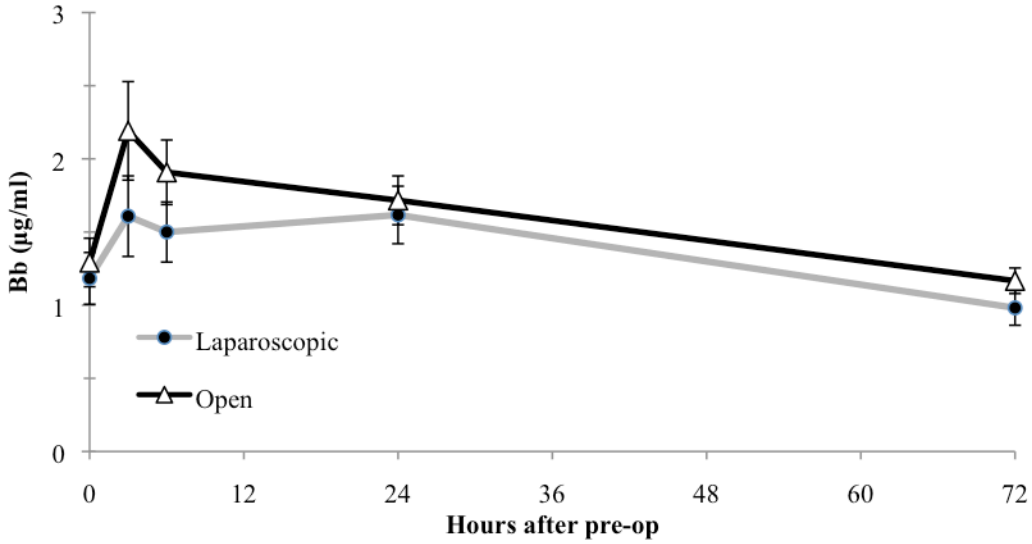


Fig. 10. Mean plasma concentration of terminal complement complex TCC by type of surgery and hours after pre-op.

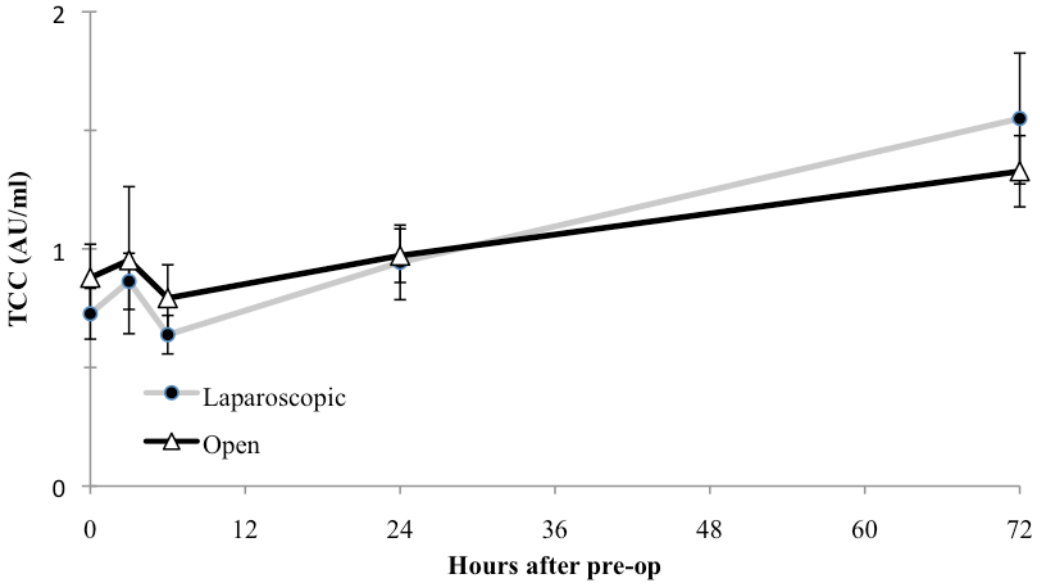


Fig. 11. Mean plasma concentration of C3bc by type of surgery and hours after pre-op.

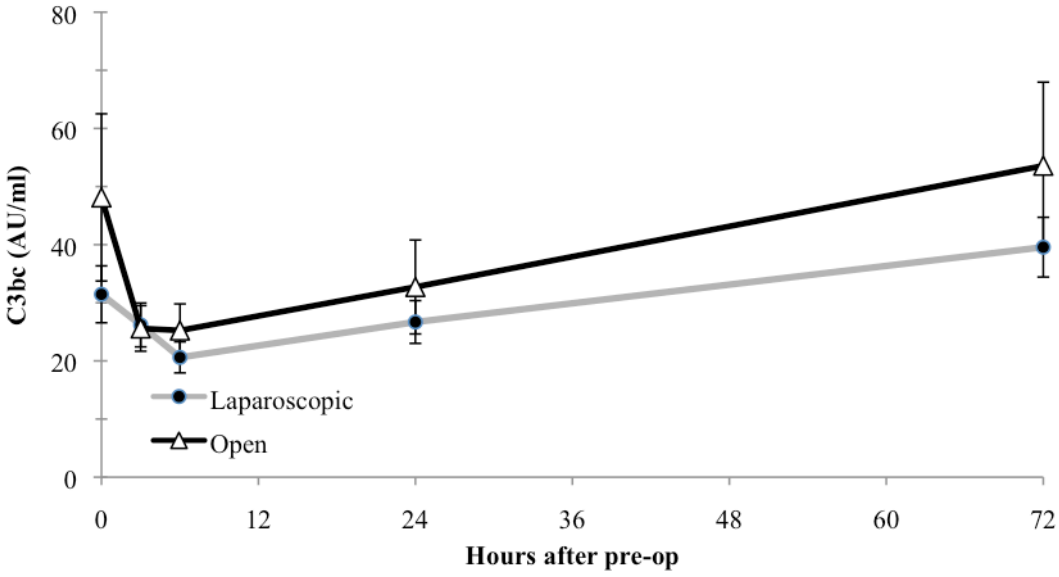
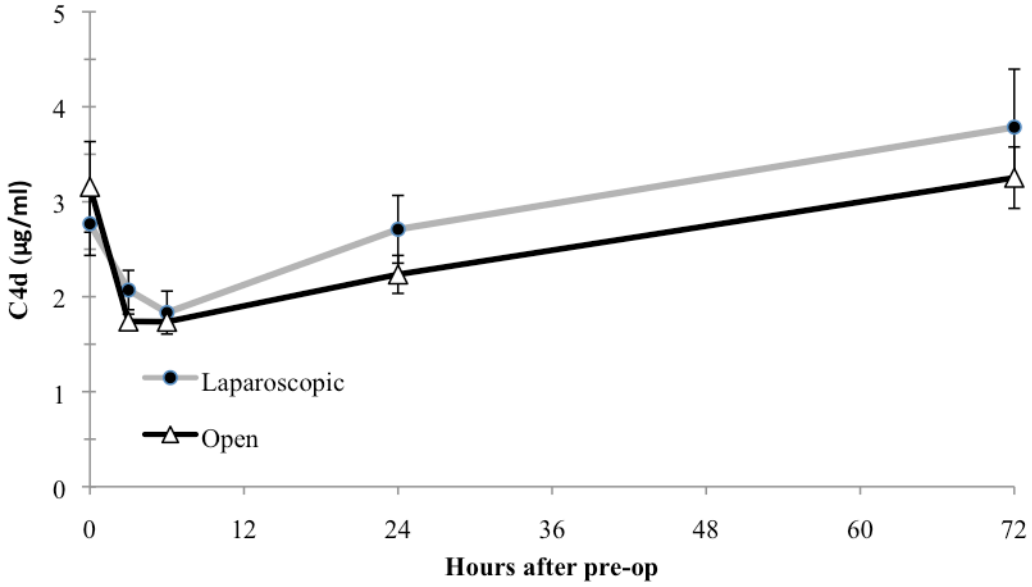


Fig. 12. Mean plasma concentration of C4d by type of surgery and hours after pre-op.



Study 4

There was no significant difference between groups regarding age, sex, type of procedure (anterior resection or abdomino-perineal resection) or length of hospital stay postoperatively. There was a difference regarding peri-operative bleeding, duration of surgery and anesthesia. In the laparoscopic group the median bleeding was 200 ml and 1150 ml in the open group. The median duration of surgery was 340 minutes in the laparoscopic group and 250 minutes in the open group. The median duration of anesthesia was 432 minutes in the laparoscopic group and 320 minutes in the open group (Table 1).

IL-1α: The median levels of the pro-inflammatory cytokine IL-1α were not altered at any time in either surgical group.

IL-6: Concentrations of IL-6 were significantly higher in the open group as compared to the laparoscopic group at T1 and T2. The mixed model analysis confirms a significant interaction effect between time and type of surgery. (p=0.003).

IL-8: The changes are similar to the changes seen for IL-6. The concentration of IL-8 is elevated compared to baseline but considerably lower in the laparoscopic group. The mixed model analysis does however not confirm a significant interaction effect between time and type of surgery (p=0.069).

IL-10: The concentrations increased at T1 and T2 in both surgery groups compared to baseline. The concentration at T1 was lower in the laparoscopic group. The mixed model analysis of data confirms a difference between groups. There is a significant interaction effect between time and type of surgery (p=0.008). The difference between IL-10 concentrations was greatest at T1.

TNF-α: The median concentrations of TNF-α at T1 and T2 were slightly increased in both groups compared to baseline values. There was not a significant difference between types of surgery (p=0.334).

CRP: The concentration increased in both groups during surgery. At T3, 24 hours after surgery started, the median concentration was 57.5 mg/l in the laparoscopic group and 100.0 mg/l for the open group. The statistical analysis showed a significant interaction between type of surgery and time (p=0.012).

WBC: After 24 hours (T3) the median WBC was increased in both groups compared to baseline values. There were no significant differences between surgical groups (p=0.794).

ICAM-1: At T1 the median concentration of ICAM-1 had decreased in the open group and increased in the laparoscopic group. The concentration of ICAM-1 then increased and reached maximum values in both groups at T4, 3-5 days after surgery. There was a significant interaction between surgery and time (p=0.010).

VCAM-1: The concentration of VCAM-1 increased during surgery and in the early postoperative phase in both groups. The highest concentrations were measured at T4 in both groups. There was no significant interaction effect between surgery and time (p=0.956).

Fig. 13. Mean plasma concentration of IL-6 by type of surgery and hours after pre-op

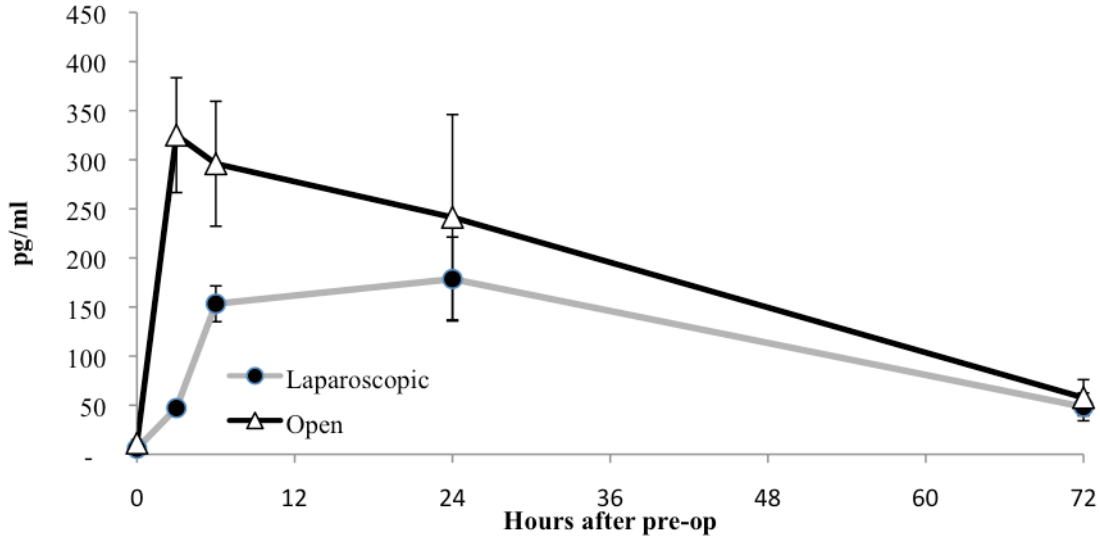


Fig. 14. Mean plasma concentration of IL-8 by type of surgery and hours after pre-op.

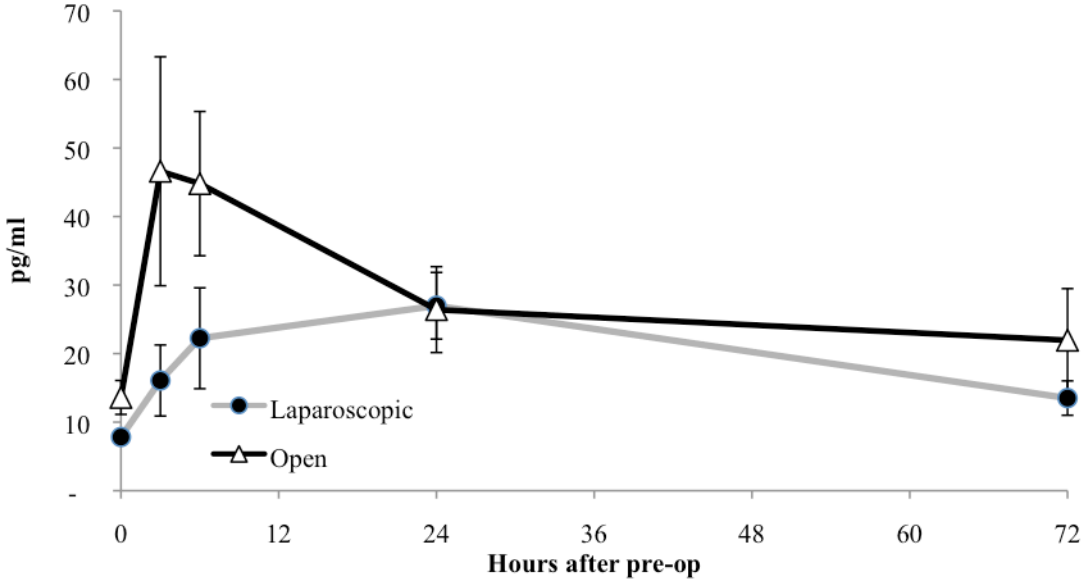
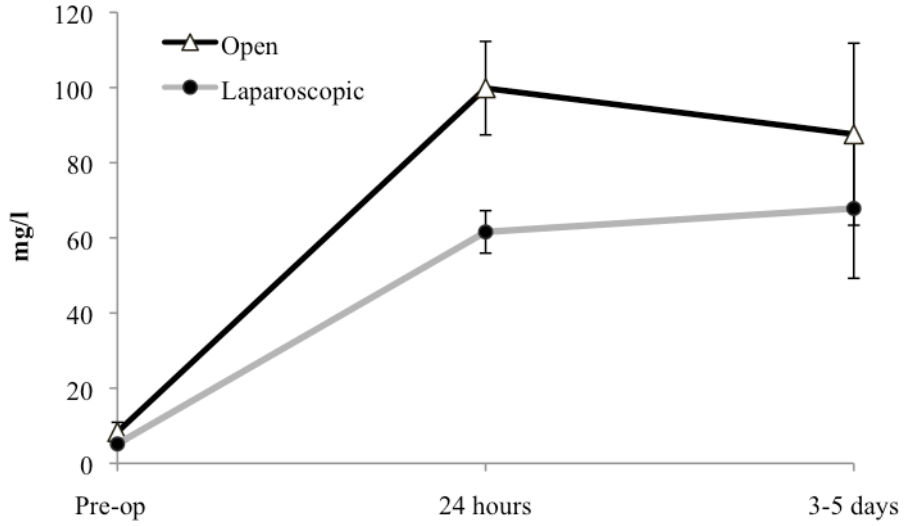


Fig. 15. Mean plasma concentration of CRP by type of surgery and hours after pre-op.



Discussion

Modulation of inflammatory response in intra-operative blood salvage by heparinization of tubing.

The study presented in paper 1 shows that intra-operatively salvaged blood contains elevated concentrations of complement split products and pro-inflammatory cytokines. It also indicates that heparin coating of surfaces does not decrease the levels of complement split products and pro-inflammatory cytokines in salvaged blood compared to non-heparin-coated surfaces. It has been shown in other types of extracorporeal circulation that a heparin-coated perfusion system lowers the concentration of complement split products.[41] In vitro-studies by Lappegård et al shows that polyvinyl chloride (PVC) coated with heparin inhibits complement activation.[75] In their whole-blood model they compared circulation of blood in tubing with and without heparin-coating. Activation of complement and release of chemochines were inhibited by heparin coating.[76] They also showed that the release of chemochines and activation of leukocytes is largely complement dependent.[77] In our study we found elevated levels of complement split products and interleukins. This suggests that heparin coating of PVC does not affect complement activation caused by surgical trauma, ischaemia-reperfusion and blood-gas interaction.

In orthopedic surgery, studies have reported levels of C3a as high as 3000-4000 ng/ml in postoperatively salvaged shed blood. We detected elevated levels of complement split product in our study but considerably lower than in previous studies. The levels of C3a were elevated in shed blood collected by both tubing systems. The median concentrations of C3a were around 700 ng/ml in both groups. Regarding pro-inflammatory cytokines, the concentrations of IL-6 and IL-8 were elevated in intraoperatively salvaged blood in our study, described in paper 1. Compared to postoperatively salvaged blood the levels were lower.[37] Intraoperatively salvaged blood is transfused back to the patient earlier than

postoperatively salvaged blood. Therefore, the blood is exposed to tissue factors, air, foreign surfaces etc for a shorter time. This may be an explanation to why we found lower levels of pro-inflammatory cytokines and complement split products.

As a sign of complement system activation, the anaphylatoxin C3a is formed in association with the collection of blood. Earlier studies regarding postoperative salvage have demonstrated elevated complement split products in salvaged blood.[78] Compared to concentrations of C3a found in postoperative salvage, the concentrations were lower in intra-operative salvaged blood.[79]

The level of the enzyme PMN-elastase was significantly elevated in salvaged blood. Studies of postoperative salvage have also confirmed an increase of PMN-elastase in salvaged blood.[37] No significant difference was found between the heparin-coated group and the non-heparin-coated group. Studies of cardiopulmonary bypass systems have indicated a decreased level of PMN-elastase in blood circulated in biocompatible circuits coated with heparin.[41] Biochemical experimental studies suggest that elastase activity is inhibited by heparin.[80]

No negative clinical effects have been shown at postoperative transfusion of the blood to orthopedic patients. It is assumed that giving back large volumes of shed, filtered blood, more than 1500 ml, may be harmful and may cause anaphylactic reactions. The data in this study show that intra-operative blood is less activated than blood collected postoperatively.

The results of the present study indicate that the blood salvaged intra-operatively contains elevated levels of complement split product and pro-inflammatory cytokines and that heparin-coated surfaces of the salvage device do not significantly influence the formation of inflammatory mediators.

Modulation of stress response by anesthesia

The results in paper 2 showed that levels of IL-6 were elevated already 30 min after end of surgery. In patients anesthetized with propofol-remifentanil (TIVA) the median concentration was 1770 pg/ml and in the patients anesthetized with sevoflurane 1515 pg/ml. The concentrations of IL-6 remained elevated at 24 hours after surgery but at lower levels. The main results from our study show that there is a pro-inflammatory response in patients who are subject to major colorectal surgery with release of IL-6 and IL-8 in the early post-operative period. The type of anesthesia that was used did not significantly affect the pro- and anti-inflammatory response or complement activation. Regarding the anti-inflammatory response, our study shows that there is release of IL-10 in these patients after surgery.

The effect on the systemic inflammatory response by two different anesthetics (propofol-remifentanil and sevoflurane) was one of the aims described in paper 2. We hypothesized that colo-rectal surgery is a good model for this investigation since it is significant surgical trauma and the duration is several hours, and thereby several hours of anesthetic exposure. However, we could not detect a difference regarding levels of interleukins and complement split products. Compared to the effect of the surgical trauma itself the effect of anesthesia on inflammation is probably quite small. Our data show that there is an inflammatory response with elevated levels of pro-inflammatory cytokines during colorectal surgery and in the early postoperative period. Similar levels of IL-6 were found peri-operatively in patients randomized to propofol-remifentanil TIVA or sevoflurane VIMA during hysterectomy.[81] Patients undergoing cholecystectomy were randomized to TIVA with propofol and remifentanil or inhalation anesthesia with isoflurane.[82] In accordance with our findings they also detected elevated levels of IL-6 in the early postoperative period in both groups. However, in their study, the levels of the pro-inflammatory cytokines IL-6 and TNF-α were higher in the isoflurane group compared to the group where the patients received propofol and remifentanil.[82] Since isoflurane and sevoflurane are both halogenated volatile anesthetics one

could expect similarities also in how they affect inflammation. Some years ago Crozier and colleagues found that propofol-alfentanil anesthesia causes a decreased pro-inflammatory response with lower levels of IL-6 as compared with patients anesthetized with isoflurane.[45] They suggested that this was an alfentanil-mediated effect on opioid receptors which leads to reduced intracellular cyclic adenosine monophosphate (cAMP). This second messenger mediates release of IL-6.[45]

In a study by El Azab and colleagues patients subjected to coronary artery by-pass surgery (CABG) were randomized to volatile induction anesthesia with sevoflurane, TIVA with propofol or midazolam/sufentanil. Similarly to this study they did not find a difference in TNF-α, IL-6 or IL-8 between the groups during surgery or in the postoperative period. There was an elevated concentration of IL-6 in the sevoflurane group after induction of anesthesia but before start of cardiopulmonary bypass compared to the two TIVA-groups.[83]

Gilliland and colleagues found an increased anti-inflammatory response with higher levels of IL-10 in patients receiving propofol-alfentanil anesthesia compared to inhalation anesthesia with isoflurane.[84] Opposite results were published by Schneemilch et al who found higher postoperative values of IL-10 in patients undergoing minor surgery who received balanced inhalational anesthesia with sevoflurane compared to propofol and alfentanil.[85] Our results do not verify this difference between different types of anesthesia regarding concentrations of IL-10. There is evidence that the anti-inflammatory cytokine IL-10 response is of importance in patients subject to major abdominal surgery. In a study by Dimopoulou and colleagues the IL-10/TNF-α quotient was correlated with the occurrence of post-operative complications.[86] Interleukin-10 has anti-inflammatory abilities and inhibits the synthesis of pro-inflammatory cytokines.[32] IL-10 shifts the immune-response from Th1-type to Th-2 type (19).[87] In colorectal cancer patients there are decreased levels of CD4+ Th1-type cells and increased levels of IL-10. High serum levels of this cytokine are considered to be a negative prognostic factor

for disease-free intervals and overall survival.[88] Volatile anesthetics affect the intra-cellular calcium-metabolism and cause a rise in cytosolic Ca^{2+} concentrations (21).[89] Human cells cultured in an environment with high calcium concentrations increase their production of IL-10.[90]

Major colorectal surgery activates complement as measured by elevated levels of C3a per-operatively and after 24 hours post-operatively. In paper 2 there was an activation of complement with elevated levels of the split product C3a. Our results show that the extent of complement activation is the same regardless of which anesthetic that is used (sevoflurane or propofol). The study also shows that complement is activated intra-operatively and in the early post-operative period.

Laparoscopic rectal surgery and inflammatory response

Surgical trauma induces an inflammatory response with release of interleukins. The magnitude of the systemic inflammatory response has been correlated to the extent of tissue trauma. In minor surgery the release of inflammatory mediators is mainly local. In major surgery, a systemic inflammatory response can cause organ dysfunction and eventually multiple organ failure. It is therefore of interest to find techniques in anesthesiology, surgery and perioperative care that modulate the inflammatory response in a favorable way. The results described in paper 2 shows that there is a significant increase of pro-inflammatory cytokines during colorectal surgery. We found similar results in our next study described in paper 4. The levels of IL-6 were increased compared to baseline 180 minutes after start of surgery in both groups. The levels of IL-6 were 45.6 pg/ml in the laparoscopic group and 276.8 pg/ml in the open group. The level of IL-6 was significantly lower during laparoscopic surgery as compared to open surgery. This supports the hypothesis that laparoscopic surgery causes a less pronounced stress response due to less tissue trauma. That laparoscopic rectal surgery actually causes a lesser stress response is

also supported by the results of C-reactive protein. In the same study (paper 4) CRP was measured at three separate time-points before and after surgery. The levels of CRP were elevated 24 hours after surgery in both groups. The concentration of CRP was significantly higher in the patients who underwent open surgery. Our data suggest that laparoscopic surgery causes a less pronounced inflammatory response as compared to open rectal surgery.

Our findings of lower release of IL-6 in laparoscopic compared to open colorectal surgery confirm results from other studies.[55,91] The concentration of IL-6 is also elevated when complications occur such as postoperative sepsis and anastomotic leakage.[92,93]

It has been suggested that the lesser degree of inflammatory response in laparoscopic surgery could have a positive oncologic effect.[64] In colorectal cancer the host is increasingly immuno-suppressed as the disease progresses. It could be helpful to preserve immunity in patients whose immunity is already compromised by malignant disease. The immune-response is shifted from a Th1 to a Th2 type response.[94] The shift from a cell-mediated response to a humoral response increases circulating levels of cytokines such as IL-4, IL-6 and IL-10. Increased levels of IL-10 have been associated with less responsiveness to treatment and a shorter survival for patients with colorectal cancer. Whether the extent of the surgical trauma and its immunological consequences really has an impact on the cancer disease is not fully known. Studies by Lacy et al on laparoscopic vs. open colectomy due to cancer shows a better long-term survival in the laparoscopic group.[95] Our study also showed a significantly lower level of CRP in the laparoscopic group. This finding has also been shown in other studies. CRP is an acute phase protein and is elevated after trauma and infection. CRP might be a risk factor for development of colon- but not rectal cancer.[96]

The duration of surgery was significantly longer in the laparoscopic group. The fact that laparoscopy is less invasive and causes less tissue trauma seems to be more

important than the duration of surgery. The result in study 3 indicates that rectal surgery causes an activation of the complement system. Complement was activated through the alternative pathway as Bb was increased and C4d was not. The activation of complement was similar in the two treatment groups. Traumatic tissue injury can induce activation of complement through the alternative pathway.[97] In a study by Ellström et al., complement activation was measured during abdominal and laparoscopic hysterectomy.[98] They found no signs of complement activation in either of the groups. Equal inflammatory response in both laparoscopic and open surgery has been found in studies comparing relatively minor procedures, such as inguinal hernia repair, where the surgical trauma is minor.[61]

Future perspectives

Cytokines and other markers of the inflammatory response to surgical trauma increase during and after surgery. The pro-inflammatory cytokine IL-6 reflects the degree of injury severity in surgery, trauma and intensive care.[28] Analysis of IL-6 and other cytokines can be performed today in many hospital laboratories. However, the use of IL-6 in clinical practice is not widely spread even though equipment for point-of-care analysis of IL-6 is already available.[99] Analysis of IL-6 could be integrated into the laboratory panel in intensive care units and allow stratification for clinical intervention. Monitoring of IL-6 and IL-8 is used in neonatal care for early detection of sepsis.[100,101] Point-of-care devices that give a wider monitoring of the inflammatory response are being developed. A protein chip for parallel quantification of IL-6, IL-8, IL-10, TNF-α, S-100, PCT, E-Selectin, CRP and neopterin has been presented. Only 4 μL of patient serum is required and the process takes 2.5 hours.[102] Several therapeutic anti-bodies directed at cytokines and the complement system have been introduced with various results.[103] In clinical trials IL-6 has been used to stratify septic patients for treatment with monoclonal anti-TNF antibodies.[104,105] In the future it will be increasingly important to monitor markers of the systemic inflammatory response as new specific antibody therapies

are being developed. More research in this field is required to improve diagnostics and treatment of trauma, sepsis and other hyper-inflammatory states.

Limitations of the study

The study aims were to investigate biochemical effects of different techniques of surgery, anesthesia and autologous blood transfusion. We detected lower systemic levels of IL-6 and CRP in laparoscopic surgery compared to open rectal surgery. In the studies comparing different types of anesthetics and intra-operative blood salvage devices there were no statistically significant differences between groups regarding cytokine levels and complement activation. These results are affected by the low sample size but even with larger samples we believe that these differences would still not be of clinical significance. As previously stated, this study did not investigate clinical effects but only effects on levels of markers of the systemic inflammatory response. We believe that the measured markers are of clinical importance. Studies that aim at describing cytokine patterns and clinical outcomes are of great interest but to detect a difference between groups regarding patient morbidity the sample sizes need to be considerably higher. A multi-center study design is then needed for completion of the study in a reasonable amount of time.

Conclusions

1. Blood salvaged intra-operatively during total hip artroplasty contains elevated levels of complement split products and pro-inflammatory cytokines.

2. Heparin-coated surfaces of the salvage device do not significantly influence the formation of inflammatory mediators.

3. Major colorectal surgery leads to activation of the complement cascade and the release of both pro-inflammatory and anti-inflammatory cytokines. Complement is activated through the alternative pathway.

4. There are no significant differences between total intravenous anesthesia (TIVA) with propofol and remifentanil and inhalational anesthesia with sevoflurane and fentanyl regarding complement activation and the release of pro- and anti-inflammatory interleukins.

5. Rectal surgery causes release of both pro- and anti-inflammatory cytokines. The inflammatory response is lower in laparoscopic rectal surgery as compared to conventional open surgery.

Acknowledgements

I wish to express my sincere gratitude to the following persons who contributed to this thesis:

Anders Bengtsson and **Jan-Peter Bengtson**. My scientific tutors at Department of Anesthesiology and Intensive Care Medicine. Thank you for all your help, guidance and friendship over the years. I am deeply grateful that you gave me the opportunity and inspired me to try research.

Roman Sarbinowski, Lena Jacobsson, Torbörn Swartling and **Göran Kurlberg**. Co-writers and colleagues at Sahlgrenska University Hospital/East.

Tom-Eirik Mollnes and **Andrei Sokolov**. Co-writers from Department of Immunology, Rikshospitalet University Hospital, Oslo, Norway. Many thanks for all your help with analyses of blood samples. Thank you for having me as a guest in your laboratory and learning me about ELISA-methods.

Maria Tylman. Thank you for skilful laboratory assistance.

Christina Raner, Olof Ekre and **Åsa Haraldsson**. Former and present heads of Department of Anesthesia and Intensive Care, Sahlgrenska University Hospital/East. Thank you for always being positive about my project and for giving me the opportunity to work on this thesis.

Björn Biber and **Sven-Erik Ricksten**. Former and present professors and academic heads of the department of Anesthesia and Intensive Care, The Sahlgrenska Academy, University of Gothenburg.

Hannele Magnusson. Thank you for always helping me out, both with small things and the most important ones.

Caterina Finizia. Head of the clinical internship program at Sahlgrenska University Hospital. Thank you for giving me the opportunity to combine clinical internship and PhD-studies.

Arvid Otterlind. Pediatric anesthesiologist who was my mentor when I was a medical student. Thank you for being a positive role model and for showing me the every-day of an anesthesiologist. If it wasn't for you I would probably have ended up elsewhere in medicine.

Ann-Louise Helminen and **Hillevi Björkqvist**. Thank you for your help with collection and handling of blood samples.

Thomas Marlow. Statistician. Department of Neuropsychiatric Epidemiology, Sahlgrenska Academy. For your patience, expertise and skilful guidance in the mysteries of statistics and computers. I am also thankful for your help with corrections of the English language during the writing process.

This work was funded by government funds under the **LUA/ALF agreement** and by the **Gothenburg Medical Society.**

Family, friends and all my **colleagues at Department of Anesthesia and Intensive Care, Sahlgrenska University Hospital,** for encouragement and support.

My wife **Ina**, for all your love, patience and support over the years.

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