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**Inflammatory Response in
Minor & Major Surgical Procedures**

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Abstract

Surgical procedures promote an inflammatory response, correlating to the extent of the procedure performed and influencing outcome after surgery. The aims of the thesis were to investigate factors modulating the inflammatory response perioperatively and to evaluate cell salvage for indications so far not accepted in clinical practice.

Material and Methods: In four prospective studies, inflammatory mediators were investigated perioperatively. I: The inflammatory response to 3 methods of breast reconstruction differing in complexity and the use of silicone implants in an otherwise identical patient population was determined. II: In 18 patients undergoing orthotopic liver transplantation (OLT) the activation and release of pro-inflammatory mediators was compared in patients receiving a liver with a cold ischemia time (CIT) > 12 hours versus a CIT < 12 hours. III: To reduce allogeneic blood transfusion in liver resection, the quality of intraoperatively salvaged, washed shed blood was investigated in a pilot study. The results were compared to blood salvaged and processed during aortic surgery. IV: In 24 patients scheduled for hip arthroplasty, pro-inflammatory mediators were investigated in intraoperatively salvaged, filtered shed blood. Heparin-coated tubing systems were compared to non heparin-coated tubing systems.

Results: I: IL-6 was elevated in all groups on the first postoperative day; IL-8 was significantly elevated 2 weeks postoperatively in all groups, women with silicone implants having the highest plasma concentrations. II: Plasma concentrations of C3a, C5b-9, neopterin, IL-6, and IL-8 were elevated 120 minutes after reperfusion in both groups, only IL-8 was different between groups. III: Inflammatory mediators were elevated in the salvaged blood in both groups; after the washing procedure IL-6, C3a, and C5b-9 were lower in the salvaged blood than in patients' blood in the liver resection group; contamination with intestinal flora could not be excluded in one patient. IV: C3a, sC5b-9, PMN elastase, IL-6 and IL-8 were elevated in the salvaged blood in both groups without difference between heparin-coated and non heparin-coated tubing systems.

Conclusions: Flap procedures stimulated a minor pro-inflammatory response; however, silicone implants seemed to have an immunomodulatory effect. As has been shown previously, pro-inflammatory mediators were released upon reperfusion during OLT; only IL-8 correlated to the duration of CIT. Shed blood, salvaged during liver resection, contained high levels of pro-inflammatory mediators; but after processing, it seemed to be as safe as cell salvaged blood during aortic surgery regarding inflammatory mediators; possible contamination with intestinal flora requires further thorough investigation and evaluation. Blood salvaged intraoperatively during hip arthroplasty contained elevated levels of pro-inflammatory cytokines and complement split products; however, concentrations were lower than previously reported in postoperatively salvaged and filtered shed blood, which has been successfully transfused. Heparin-coating of the tubing systems did not influence the formation of inflammatory mediators.

Keywords: inflammatory response, surgery, cell salvage, cytokines, complement

Original papers

The thesis is based on the following papers, which will be referred to in the text by their roman numerals:

- I Schmidt A, Bengtsson A, Tylman M, Blomqvist L: **Pro-inflammatory cytokines in elective flap surgery.** J Surg Res. 2007;137: 117-21.
- II Schmidt A, Tomasdottir H, Bengtsson A: **Influence of cold ischemia time on complement activation, neopterin, and cytokine release in liver transplantation.** Transplant Proc. 2004;36: 2796-8.
- III Schmidt A, Sues HC, Siegel E, Peetz D, Bengtsson A, Gervais HW: **Is cell salvage safe in liver resection? A pilot study.** J Clin Anesth. 2009;21: 579-84.
- IV 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: 33-8.

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Abbreviations

AS	aortic surgery
ANOVA	analyses of variance
C	complement
CIT	cold ischemia time
CS	cell saver
C3a	activated complement split product 3, anaphylatoxin
C5a	activated complement split product 5, anaphylatoxin
C5b-9	terminal complement complex = membrane attack complex
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
HIV	human immunodeficiency virus
IFN	interferon
IL	interleukin
I/R	ischemia / reperfusion
LD	latissimus dorsi flap
LPS	lipopolysaccharide
LTD	lateral thoracodorsal flap
LS	liver surgery
OLT	orthotopic liver transplantation
PMN	polymorph nuclear granulocyte
RBC	red blood cell
SARS	severe acute respiratory syndrome
SIRS	systemic inflammatory response syndrome
TNF	tumor necrosis factor
TRAM	transverse rectus abdominis muscle flap

Introduction

Surgical procedures promote an inflammatory response as part of the host's defense mechanism to trauma. The extent of immunomodulation after surgery mainly correlates to the tissue trauma encountered [1, 2]. But not only the procedure itself contributes to the inflammatory response, immunomodulation after surgery is also influenced by a variety of parameters including the patient's medical history, duration of surgery, or the use of extracorporeal circulation [1, 3, 4]. In recent years, it has been shown that blood transfusions themselves modulate the immune response, the age of the blood product and the number of units transfused being of major concern. In critically ill patients, blood transfusion has even been correlated to poor outcome [5, 6]. This stimulated clinicians and researchers alike to find alternative treatment options.

Regardless of the triggering insult, the immune response to trauma follows a rather stereotypical pattern, involving different cell types, pro- as well as anti-inflammatory mediators. The purpose of the inflammatory response to surgery is, first, to defend the organism against invading pathogens, and, second, to organize wound healing. In minor tissue injury, modulation of the immune system is restricted to the site of trauma [7]. However, the immune reaction to major trauma and surgery involves exaggerated release of mainly pro-inflammatory mediators like interleukin (IL)-1, IL-6, IL-8, and IL-18, tumor necrosis factor (TNF)- α , neutrophil activation, and uncontrolled neutrophil and macrophage oxidative burst [8]. These mediators can be found in the circulation and may promote systemic inflammatory response syndrome (SIRS) and organ failure.

Biomarkers like cytokines and complement split products, released during surgery and trauma, correlate to the severity of the threat encountered. Thus they provide useful information in evaluating the inflammatory response to certain interventions, as well as in assessing and stratifying new therapeutic options to established therapies.

Reconstructive surgery

Primarily, plastic surgery is not vital and does not improve survival, but might influence psychological well-being. Most procedures are of short duration, and include skin and its

layers only. Reconstructive surgery may be more complex, involving soft tissue as well as autologous grafts and foreign material for tissue augmentation.

Cancer disease itself modulates the immune system [9]. In case of reconstructive surgery at the site of former cancer operations and radiation, the cosmetic result as well as the immune response to the procedure may differ from those of otherwise healthy patients. Additionally, foreign material has been shown to modulate the immune system [10, 11], but its impact on long-term results is still controversial [11-13]. Therefore, we wanted to compare the immediate inflammatory response to three different surgical techniques for delayed breast reconstruction after mastectomy for breast cancer. The procedures performed differed in complexity and the requirement for silicone implants. Patients' satisfaction and quality of life were studied earlier and have been shown to be comparable in all groups [14] (**paper I**).

Cold ischemia time

During liver transplantation (OLT) plasma levels of pro-inflammatory mediators have been found to be very high [15, 16]. This can be attributed, at least in part, to the procedure itself, being one of the most traumatic abdominal operations performed. However, the immune system in patients with end stage liver disease is known to be modulated, either by an underlying infectious disease or as a result of fibrotic remodeling of the cirrhotic liver. Monocytes from cirrhotic patients produce more TNF- α and IL-6 upon lipopolysaccharide (LPS) challenge than monocytes from normal controls [17], and at the same time they are defective in releasing anti-inflammatory mediators like IL-10 [18]. This suggests that, at least during the initiation of host defense, the immune response is prevailed by a pro-inflammatory pattern of mediators in end stage liver disease.

In liver transplantation, one major source of cytokine release and complement activation is the grafted liver itself. Sinusoidal epithelial cells are most affected during cold storage, whereas Kupffer cells, resident hepatic macrophages, are activated upon reperfusion, followed by neutrophil activation, and finally the release of pro-inflammatory mediators [19-21]. Among other factors, the duration of cold ischemia time (CIT) has been

identified to affect graft and patient survival after OLT, especially in combination with older donor age [22-24]. Recently, a large retrospective study confirmed that livers transplanted with a CIT > 12 h were associated with an overall higher risk of graft loss when compared to those with a CIT < 12 h, but it also identified recipient subgroups with high susceptibility to prolonged CIT. Recipient diabetes and obesity, and donor African American ethnicity were found to significantly amplify the adverse effects of CIT > 12 h. In contrast, in non-obese and non-diabetic recipients CIT did not influence outcome after OLT [25]. The influence of the duration of cold ischemia time on inflammatory mediators was not specifically investigated. We wanted to investigate whether duration of CIT has an impact on the release of pro-inflammatory mediators, and activation of the complement cascade in patients undergoing OLT. This might explain the impaired graft survival as well as hemodynamic alterations observed after reperfusion in a subset of patients 8 (**paper II**).

Cell salvaged washed blood

Blood is so far the only worldwide available, approved option to treat severe anemia in the emergency setting. But besides its therapeutic properties, allogeneic blood transfusion exposes patients to the risk of allergic and infectious disease, immunomodulation, and acute lung injury [26-28]. Consequently, a variety of alternatives to allogeneic blood transfusion have been developed. Intraoperatively, transfusion of autologous blood is the most commonly technique used, including preoperative donation, acute normovolemic hemodilution, and perioperative cell salvage of shed blood [29]. In intraoperative cell salvage, shed blood from the surgical field is collected, anticoagulated, processed, and finally returned to the patient. The commercially available devices can be subdivided into two major groups, depending on the extent to which the salvaged blood is processed prior to transfusion.

In “washed autotransfusion systems” like the cell saver (CS), the plasma is separated from red blood cells (RBC) by centrifugation after collection in the reservoir. In a second step, the remaining red blood cells are washed with normal saline to remove activated coagulation factors, cellular stroma, and pro-inflammatory mediators. The technique is well established, safe and cost-effective [30-32], but has some major limitations.

Contraindications to cell salvage include infection at the site of surgery, contamination with pro-inflammatory triggers, or malignancies [33]. In cancer surgery different approaches to eliminate viable tumor cells before transfusion have been studied, but neither irradiation nor filtration yielded convincing results so far [34-37]. Bacterial contamination of salvaged and processed blood is a known phenomenon even in so called "sterile" operations. The bacteria separated either comprise commensal skin microflora, or are airborne bacteria from the operation room in small numbers [38, 39]. In contrast, challenging the collected blood with intestinal microflora or contaminating it with bile, as might happen in liver resection, could trigger the release of inflammatory mediators in the reservoir and subsequently promote the development of SIRS in the patient after transfusion. We therefore investigated the quality of cell salvaged blood in liver resection, a procedure with a high risk for major bleeding, today not deemed acceptable for transfusion. The results were compared to patients undergoing aortic repair, where cell salvage is well established and accepted, and is included in many anesthesia protocols [40, 41] (**paper III**).

Cell salvaged filtered blood

In "filtered" autotransfusion systems like Sangvia[®] the shed blood is collected in the same way as in "washed" autotransfusion systems as described above. But instead of centrifugation and washing the salvaged wound blood it is filtered via a 200 micron filter prior to transfusion (figure 1). Experiences with this technique are mainly based on postoperative salvage. High concentrations of pro-inflammatory cytokines and complement split products were found in collected shed blood that could not be reduced through filtration [42, 43]. After transfusion, inflammatory mediators determined in plasma were elevated for a short period of time only, without any signs of systemic infection [44, 45]. It has been shown, that postoperative transfusion of filtered shed blood reduces the need for allogeneic blood transfusion, is easy to perform, and cost-effective [46-48]. These characteristics could make filtered autotransfusion systems like Sangvia[®] a useful intraoperative tool in procedures with a limited blood loss, where the CS is generally not used. But as the blood collected is not centrifuged and washed as in the CS, the concentration of inflammatory mediators is of concern, limiting the amount to be transfused to 1.5 l for safety reasons. Foreign material like the cardiopulmonary

bypass circuit can promote complement activation and cytokine release, as well as activation of the coagulation cascade. Heparin coating of the surfaces has been shown to enhance biocompatibility, and especially to reduce the release of pro-inflammatory mediators [49, 50]. As the Sangvia[®] system is so far only approved for postoperative collection of blood sampled from surgical drainage, we conducted this study to investigate the quality of intraoperatively salvaged blood with respect to the formation of pro-inflammatory cytokines and complement split products. Additionally we were interested whether heparin coating of the tubing system enhances the biocompatibility and thus reduces the release of inflammatory mediators (**paper IV**).

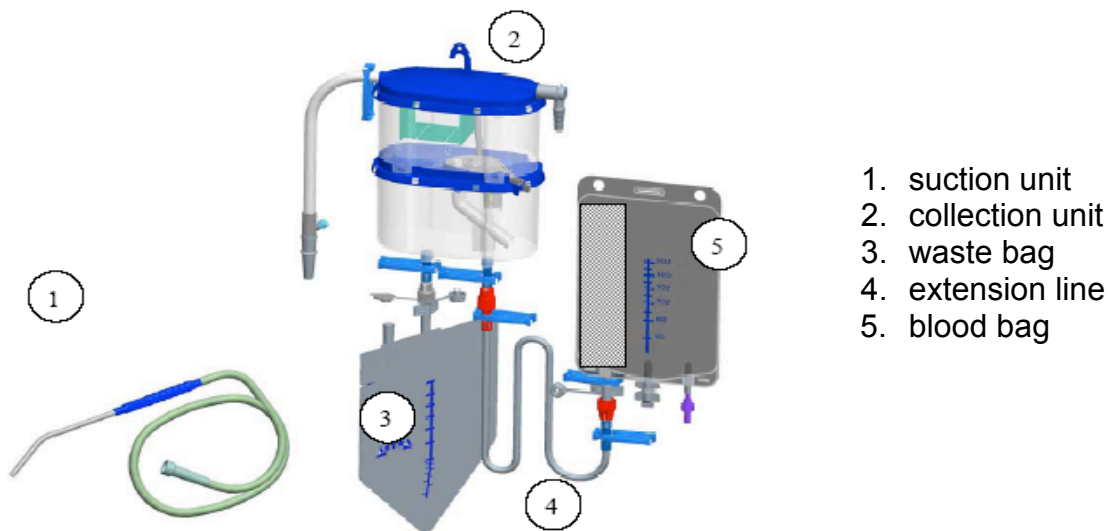


Figure 1: Schematic figure of the cell salvage device Sangvia[®].

Immunocompetent cells

Macrophages are innate immune cells that play an important role in activation of the immune response and wound healing [51]. Maturing from circulating monocytes, they are resident in almost all tissues, making them the first cells recognizing an invading pathogen as non-self. Activated by surface receptors, they eliminate the pathogen by phagocytosis and release cytokines to attract more macrophages, plasma proteins, and

especially neutrophils to the site of inflammation, thus perpetuating a positive inflammatory feedback loop [40]. A second subset of macrophages appears later in the process of inflammation, mediating tissue repair and regeneration [52].

In humans, **neopterin**, a pteridine derivative, is produced by monocytes / macrophages upon stimulation [53]. Neopterin synthesis is mainly stimulated by Interferon (IFN)- γ . Because IFN- γ is released by activated helper T-lymphocytes type 1 and natural killer cells, neopterin is regarded a sensitive marker of cell mediated immunity [54]. It is biologically stable, making the molecule an ideal marker for disease screening and monitoring. Since long, its role in the prediction of long-term prognosis in both patients with cancer [55] and those with systemic infections such as human immunodeficiency virus (HIV)-1 [56] or severe acute respiratory syndrome (SARS) [57] has been established. Recently, elevated neopterin concentrations have been reported in patients with coronary artery disease, and it was shown that increased neopterin concentrations are an independent marker for cardiovascular disease and a predictor of future cardiovascular events in patients with coronary artery disease [58, 59].

Granulocytes, especially the subset of neutrophils (PMN), are the second subtype of phagocytotic cells in the immune system. In contrast to macrophages, neutrophils are not present in healthy tissue, but circulate terminally differentiated in the bloodstream in abundance. In case of an inflammatory stimulus, neutrophils are attracted from peripheral blood by chemotactic molecules, such as complement 5a (C5a), IL-8, or bacterial peptides. Until a few years ago, neutrophils were thought to achieve their antimicrobiological properties by means of phagocytosis and degranulation only. Recently, a third mechanism was discovered: dying neutrophils can produce extracellular structures called neutrophil extracellular traps (NET) to capture and finally destroy microorganisms [60]. Besides destroying invading pathogens, numerous communications between neutrophils and T-cells have been identified, linking innate immunity to adaptive, cell mediated immune responses [61]. By attracting more macrophages to the site of inflammation and activating monocyte derived dendritic cells [62], neutrophils are regarded the main protagonists in innate immune response.

PMN-elastase, a serine protease, is stored in azurophil granules in circulating neutrophils. Neutrophil activation and degranulation results in the release of serine proteases into the extracellular space, where they operate as proteolytically active enzymes. PMN-elastase is capable of modulating the inflammatory response by

upregulating cytokine expression of IL-6 or IL-8, promoting the degradation of IL-1 and TNF- α , or targeting on receptor expression [63]. First studies are under way to investigate the effects of serine protease inhibitors on this exciting molecule in acute and chronic diseases, but results are heterogeneous and inconclusive so far [64]. Measuring extracellular concentrations of PMN-elastase provides general information of neutrophil activation and degranulation.

Cytokines

Cytokines are low molecular weight proteins, regulating communications between immune competent cells. The target cell may be the producing cell itself (autocrine), a cell nearby (paracrine), it may require cell-to-cell contact (juxtacrine), or it may affect cells in another organ (endocrine). Cytokines are released from nucleated cells upon stimulation, binding to specific cell surface receptors, and thus modulating the immune response within the target cell. They are involved in almost every aspect of immunity and inflammation, they support cell maturation as well as differentiation, and determine whether the immune response is cytotoxic, humoral, cell-mediated or allergic [65]. They are highly active at very low concentrations. To keep the inflammatory response localized or to terminate infection, they are tightly regulated via cell surface and soluble receptors, positive and negative feedback loops, and some cytokines exert predominately anti-inflammatory properties (see below). In case of tissue trauma, most cytokines involved in innate immunity like TNF- α , IL-1, IL-6, IL-8, IL-10, and IL-12 are released locally at the site of injury by macrophages and neutrophils.

TNF exists in two forms, TNF- α and TNF- β , both of them sharing the same cell surface receptor and similar inflammatory activities. TNF- β is less potent, not that abundant and mainly produced by T-cells, whereas macrophages are the dominant source for TNF- α . In acute inflammation, lipopolysaccharides on the cell surface of bacteria are the most powerful stimuli for macrophages to release TNF- α [66]. This results in recruitment and activation of neutrophils, mediating adhesion, chemotaxis, degranulation, and finally respiratory burst. Besides modulating immunity, TNF- α itself exerts biological activities. In the heart, TNF- α directly depresses myocardial function via a nitric oxide-dependent and a sphingosine-mediated pathway, resulting in decreased contractility, hypotension,

decreased systemic vascular resistance, and ventricular dilatation [67, 68]. Additionally, TNF- α reversibly impairs vascular barrier integrity, and may cause severe lung injury [69]. As virtually all cells in the body seem to be capable to produce TNF- α and its receptors are present in all cells, except on erythrocytes and unstimulated T-lymphocytes, this might explain the huge variety of actions TNF- α is involved in [66].

IL-6 is a mainly macrophage-derived cytokine in acute inflammation, but it is also released by hepatocytes, bone marrow cells, endothelial cells, and T-cells. In acute inflammation, its production is induced by a variety of stimuli like endotoxin, viruses, or TNF- α and IL-1. As it persists in plasma for much longer than TNF- α and IL-1 [70], IL-6 is a good marker for pro-inflammatory cytokine activation. IL-6 can be detected readily in serum and is almost solely responsible for both, fever and the acute phase response in the liver [71]. Like most other pro-inflammatory cytokines, IL-6 mediates some anti-inflammatory effects as well: IL-6 inhibits TNF- α and IL-1 synthesis, thus terminating the escalating inflammatory cascade [72]. It links innate to adaptive immunity, as it promotes B-lymphocyte maturation to plasma cells and it mediates T-cell activation, growth and differentiation [73]. Besides playing an important role in the immune modulation in acute inflammation, IL-6 has been linked to chronic inflammatory disease, and it also seems to be involved in cancer progression and aging [74].

IL-8 belongs to a subset of cytokines also known as chemokines. Their main responsibility is chemotaxis, which means directing immune cells to the site of inflammation. Although IL-8 seems not to be the first chemoattractant at the site of inflammation, it is the most important chemokine to recruit and finally activate neutrophils [72]. This explains that monocytes and macrophages represent the main cellular source for IL-8 [75], and that its synthesis can be triggered by a wide variety of pro-inflammatory stimuli including lipopolysaccharides, viruses, and cytokines like TNF- α and IL-1. Elevated plasma concentrations of IL-8 have been found in patients after minor surgical procedures [76] as well as in patients with severe trauma and sepsis [8, 77].

IL-1 exists in two forms, IL-1 α and IL-1 β , both of them binding to the same receptor. However, IL- α is a cell associated cytokine that can also act as a transcription factor, whereas IL- β is secreted into the extracellular space and seems to be involved in systemic inflammation [78]. It is another pro-inflammatory cytokine, primarily produced

by mononuclear cells upon stimulation through numerous agents including endotoxin, microorganisms, antigens, as well as other cytokines like IL-6 and TNF- α . It shares many biological activities with TNF- α and IL-6, including the stimulation of the acute phase response in the liver. Through its effects on the central nervous system it causes fever, anorexia and lethargy in acute inflammation [72, 79]. Spontaneous production is usually absent in health due to its tight control on the transcriptional and translational level [80]. After stimulation, large amounts of IL-1 β mRNA accumulate in monocytes without significant translation into the IL-1 β protein [81]. This might explain, why IL-1 β plasma levels may not be elevated, but treatment with IL-1 receptor antagonists can yield complete remission in patients with acute or chronic inflammatory syndromes. This has been demonstrated in juvenile idiopathic arthritis, and hence treatment with IL-1 receptor antagonists has become standard therapy for this group of patients [82]. However, in patients with sepsis, therapy with the IL-1 β receptor antagonists could not improve survival [83, 84].

IL-10 is a major anti-inflammatory regulator of innate and adaptive immunity. Although being produced by numerous cells in acute as well as in chronic inflammation and allergic disease, macrophages are the main source of IL-10 production [85]. A major stimulus for the release of IL-10 is infection itself, as endotoxin, catecholamines and both, IL-1 β and TNF- α may upregulate IL-10 directly [86]. IL-10 in turn suppresses TNF- α release, suggesting a negative feed-back mechanism between those two cytokines to terminate acute infection [87]. The main effect of IL-10 is suppression of macrophages and dendritic function, making it a potent anti-inflammatory cytokine. But the timing of its release and the cells expressing IL-10 seem to determine whether it resolves or maintains infection, and indeed driving it towards SIRS or sepsis [88].

Complement system

The complement system comprises a group of over 25 serum proteins and cell surface receptors interacting to recognize, opsonize and clear invading microorganisms, finally promoting innate immune functions such as inflammation and enhancing adaptive immunity [89]. These proteins are mainly synthesized within the liver as pro-enzymes and they circulate in plasma in inactive forms [90]. At the site of infection, activation of

the complement system can be accomplished via three different pathways (figure 2): the classical pathway, being triggered by antigen-antibody complexes; the alternative pathway is directly activated by surface molecules containing carbohydrates and lipids on bacterial cell surfaces; and the lectin pathway is initiated by carbohydrates, especially mannose, on bacterial cell surfaces. Activation initiates a cascade of downstream reactions, where the activated pro-enzymes are cleaved, yielding a large, enzymatic fragment, and a small protein. The large fragment specifically cleaves and activates the next pro-enzyme in the same way, thus sustaining and amplifying the immune response. All three pathways converge at the level of complement (C) protein 3, generating a protease called C3 convertase, and share the last steps of the complement cascade. C3 convertase cleaves C3 into the complement split products C3b and C3a, finally forming C5a, and the terminal complement complex, C5b-9. C3b directly binds to pathogens, opsonizes them and thus presents them to phagocytotic cells like macrophages and neutrophils [91]. The terminal complement complex binds to foreign cell membranes independent of any receptor and creates pores in the lipid bilayers of the target cell, potentially causing cell lyses by osmotic leakage [92]. The anaphylatoxins C3a and C5a are potent inflammatory mediators, being involved in the modulation of many steps of the immune response; they trigger oxidative burst in macrophages and neutrophils [93, 94], and may modulate the synthesis of IL-6 and TNF- α [95, 96]. C5a and with less potency C3a exert direct biological effects, e.g., regulating vasodilatation, and increasing the permeability of small blood vessels [97].

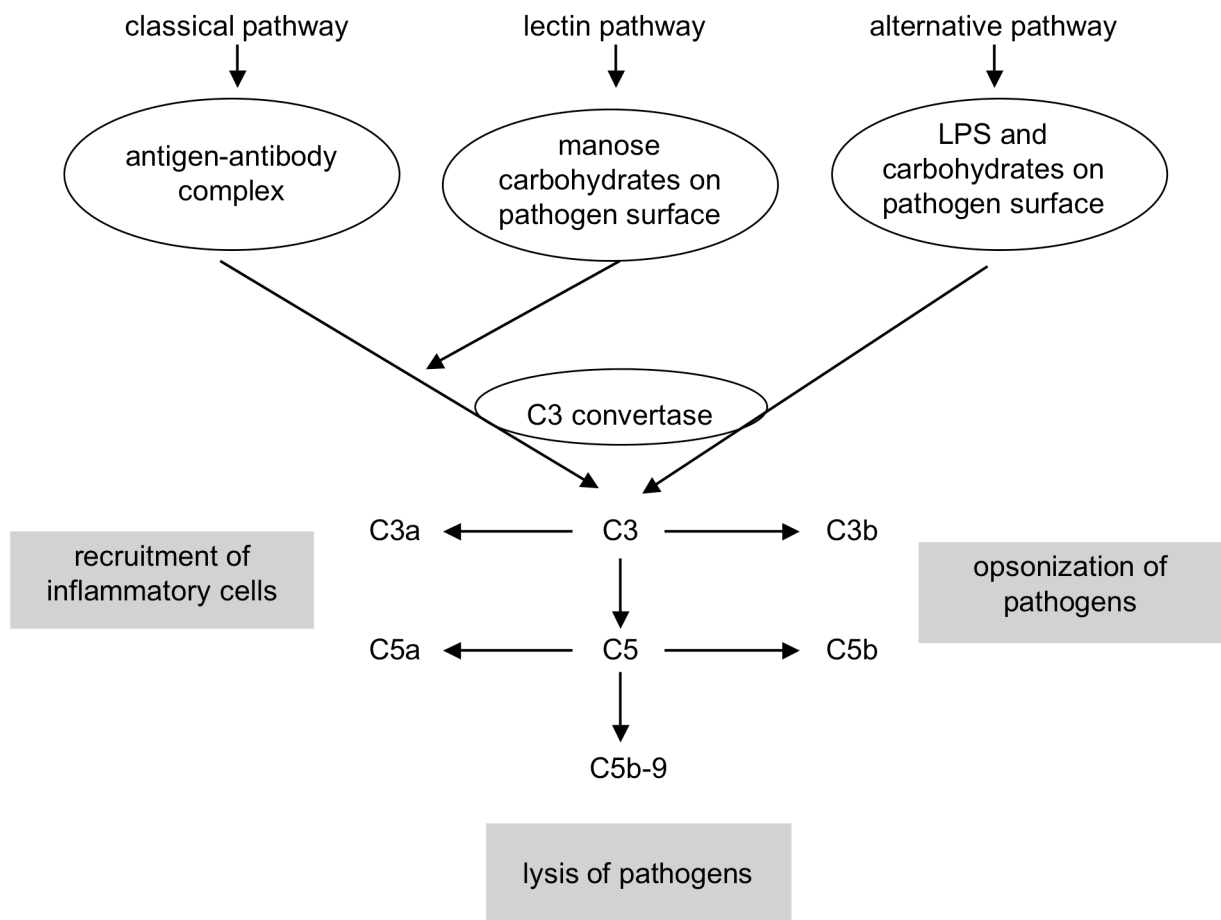


Figure 2: Schematic illustration of the complement pathways.

Aims of the study

1. To investigate monocyte / macrophage activation and the release of pro-inflammatory cytokines in three surgical procedures, differing in complexity and in the need for implantation of foreign material (**paper I**)
2. To investigate whether duration of CIT in OLT influences the activation of monocytes, the complement cascade, and the release of pro-inflammatory cytokines (**paper II**)
3. To investigate the quality of intraoperatively collected washed shed blood in liver resection surgery regarding complement activation, pro-inflammatory cytokine release, and microbiological contamination (**paper III**)
4. To investigate the quality of intraoperatively collected unwashed filtered shed blood during hip replacement surgery regarding complement activation, and pro-inflammatory cytokines (**paper IV**)
5. To evaluate whether heparin coated surfaces of the tubing system reduce the release of inflammatory mediators during cell salvage (**paper IV**)

Patients and Methods

All studies were approved by the local ethical review board responsible at the site of investigation (paper I: Karolinska Hospital, Stockholm, Sweden; paper II and IV: Sahlgrenska University Hospital, Gothenburg, Sweden; paper III: University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany). Patients were included after written informed consent was obtained.

Patients and study design

Paper I

A total of 213 women participated in a prospective, randomized, controlled trial investigating three different methods for delayed breast reconstruction after mastectomy for breast cancer [98]. The investigated procedures included the lateral thoracodorsal flap (LTD), the latissimus dorsi flap (LD), and the pedicled transverse rectus abdominis muscle flap (TRAM). Besides differing in tissue involvement, the LTD and LD require silicone implants for breast augmentation. To evaluate the inflammatory response to the different procedures, inflammatory mediators in the last 10 consecutive patients in each group were selected for analyses (figure 3).

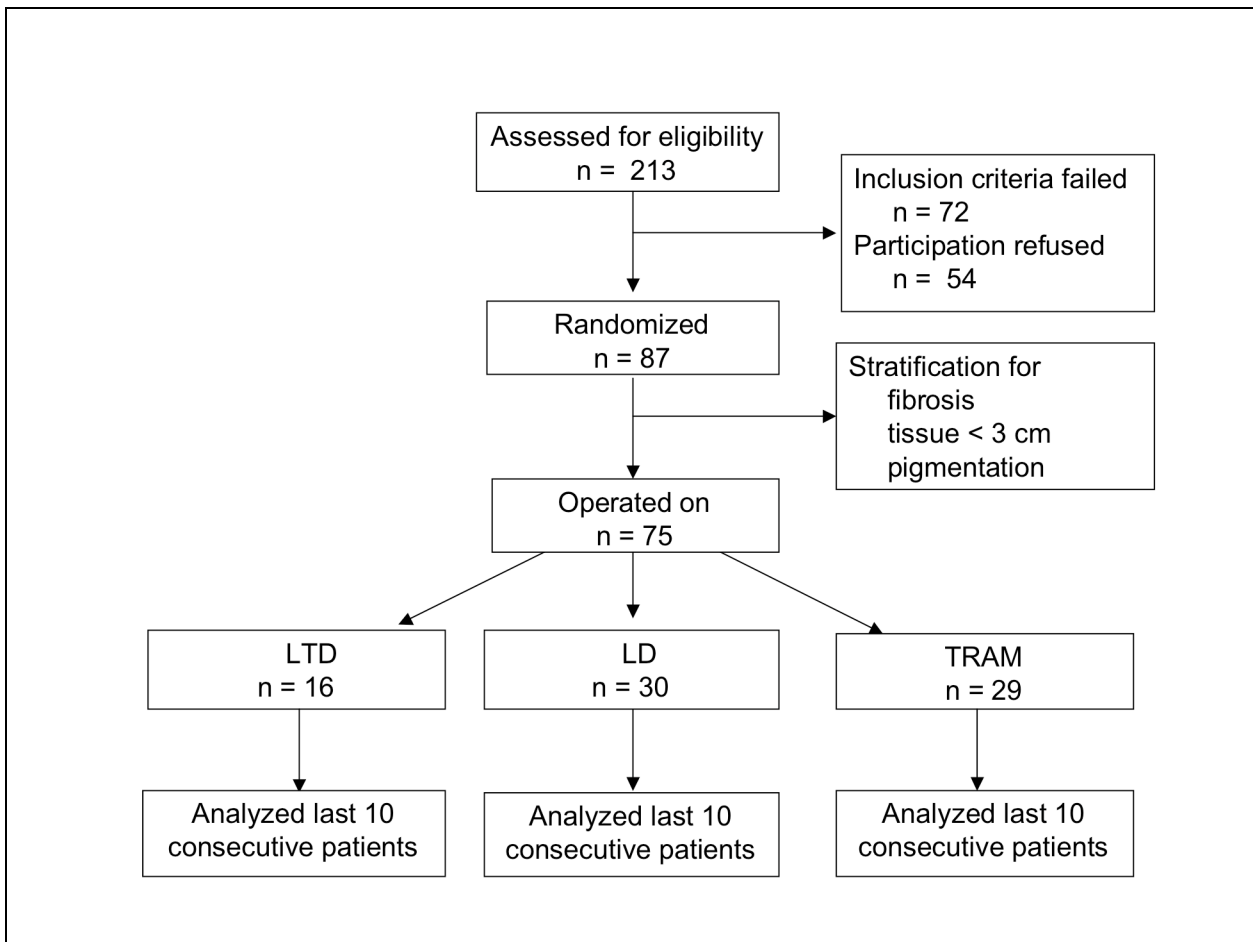


Figure 3: Patient flow in study I.

Blood samples for measurement of TNF- α , IL-6, IL-8 and neopterin were collected directly preoperatively, on postoperative day 1, and on postoperative day 14.

Paper II

Eighteen consecutive patients undergoing elective OLT were included in the study. According to the duration of CIT they were divided into two groups: CIT > 12 hours (n = 11), or CIT < 12 hours (n = 7). CIT was defined as the time from aortic clamping in the donor till portal declamping in the recipient. Immunosuppressive therapy including corticosteroids was started with induction of anesthesia. During the anhepatic phase a veno-venous bypass was established. Organs were preserved in University of Wisconsin solution and flushed with 1000 ml Haemaccel[®] (Behringwerke AG, Marburg,

Germany) prior to reperfusion. Arterial blood samples for C3a, SC5b-9, neopterin, IL-6 and IL-8 determinations were drawn preoperatively, 1 minute before and 120 minutes after reperfusion.

Paper III

Patients scheduled for hemihepatectomy (study group) or abdominal aortic surgery (control group) participated in the study. After skin incision, shed blood was collected in the reservoir of the CS (Cell Saver 5[®], Haemonetics, Munich, Germany). If blood loss exceeded 800 ml, the reservoir was attached to the CS, the blood was centrifuged, and finally washed in the automatic mode. Apart from the washing volume (1000 ml in the study group vs. 500 ml in the control group) CS settings were similar in both groups. Blood samples for determination of TNF- α , IL-6, IL-8, IL-10, and complement split products were drawn from the central venous line during the washing procedure (patient sample), from the CS-reservoir directly prior to processing, and from the washed RBC within 5 minutes after termination of the washing procedure. From the CS-reservoir and the RBC blood samples for microbiological analyses were drawn. The processed blood was not transfused to the patients in the study group.

Paper IV

Twenty-four patients scheduled for total hip arthroplasty were randomized for intraoperative blood collection with the autotransfusion system Sangvia[®] (AstraTech, Mölndal, Sweden). In group 1 (n = 12) blood was collected via a heparin-coated tubing system, in group 2 (n = 12) non-heparin-coated tubing systems were used. From patients, blood samples for determination of C3a, sC5b-9, PMN-elastase, TNF- α , IL-1 β , IL-6, and IL-8 were drawn after induction of anesthesia but before the beginning of surgery. With the beginning of surgery, the Sangvia[®] system was connected to the surgical suction device; the initial 200 ml of shed blood were collected, transferred to the blood bag and used for analyses of the respective parameters. Intraoperatively salvaged blood was not transfused to the patients.

mediators investigated	study I	study II	study III	study IV
C3a		x	x	x
C5b-9		x	x	x
TNF- α	x		x	x
IL-1 β				x
IL-6	x	x	x	x
IL-8	x	x	x	x
IL-10			x	
neopterin	x	x		
PMN-elastase				x

Table 1: mediators investigated in the respective studies.

Laboratory analyses

Inflammatory mediators

In papers I-II and IV concentrations of TNF- α , IL-1 β , IL-6, IL-8, neopterin, and PMN-elastase were analyzed with enzyme-linked immunosorbent assay (ELISA) / enzyme immunoassay (EIA) (**paper I**: TNF- α , IL-6, IL-8: Pierce Endogen, Rockford, IL, USA; neopterin: Brahms, Neuendorf, Germany; **paper II**: IL-6, IL-8: Progen Biotechnik, Heidelberg, Germany; neopterin: Henning, Berlin, Germany; **paper IV**: TNF- α , IL-1 β , IL-6, IL-8: Pierce Endogen, Rockford, IL, USA; PMN elastase: Milenia Biotec, Giessen, Germany). In **paper III** the analyses of TNF- α , IL-6, IL-8, and IL-10 was performed by means of a multiplex cytokine assay system enabling the measurement of multiple analytes simultaneously with very small sample volumes [99, 100].

In papers II-IV plasma concentrations of complement split products, C3a, and sC5b-9, were determined with commercially available quantitative ELISA / EIA kits (**study II**: C3a Progen Biotechnik, Heidelberg, Germany; sC5b-9 Behring, Marburg, Germany; **study III**: Quidel, Marburg, Germany; **study IV**: Quidel, San Diego, CA, USA).

The assays were performed according to manufacturers' recommendations and were analyzed in duplicate.

Microbiological Analyses

In **paper III** 0.5 ml of blood were cultured under aerobic conditions on Columbia blood agar plates at 37 °C for 48 h. After hemihepatectomy patient 3, the study protocol was extended; in addition to Columbia blood agar plates, Schaedler agar plates were inoculated at 36 °C for 96 h for detection of anaerobic bacteria in the last 3 patients in each group. Aerobic and anaerobic enrichment cultures were performed in the last 3 patients in each group for 6 days at 36 °Celsius (BD Bactec[®] Plus anaerobic / BD Bactec[®] plus aerobic, Becton Dickinson, Heidelberg, Germany). If bacterial growth occurred, further testing was performed following standard microbiologic techniques.

Statistics

Demographic data are expressed as median and range (**paper I-III**), or as median and 25th - 75th percentiles (**paper IV**). For statistical comparisons, a two-way analysis of variance with correction for repeated measurements (ANOVA) was performed to evaluate differences within and between groups in **paper I-II**, followed by Fisher's protected least squares difference test for multiple comparisons if intra- or inter-group results indicated a significant difference. The Wilcoxon two-sided rank sum test was used for comparisons between groups in **paper III-IV**. Differences within a group were compared with the sign test in **paper III**, and with the Wilcoxon signed rank test in **paper IV**, respectively. A probability value of ≤ 0.05 was considered significant.

Results

Paper I

Demographic data did not differ between groups. The operation times in the LTD, LD, and TRAM group were 86 min (range 73 - 157), 130 min (range 110 - 160), and 195 min (range 138 - 255), respectively.

TNF- α was not elevated at any time during the investigation. IL-6 was significantly elevated in all groups on the first postoperative day compared to preoperative values. On postoperative day 1, values in the TRAM group were significantly elevated compared to patients undergoing LD and LTD procedures (figure 4). IL-8 was significantly elevated in all groups 2 weeks after the operation compared to preoperative values, TRAM operated patients having the lowest plasma levels (figure 4). Neopterin values were within the normal range in LTD and LD operated patients at all three time points. In TRAM flap operated patients neopterin levels were significantly elevated 2 weeks after the operation (figure 4).

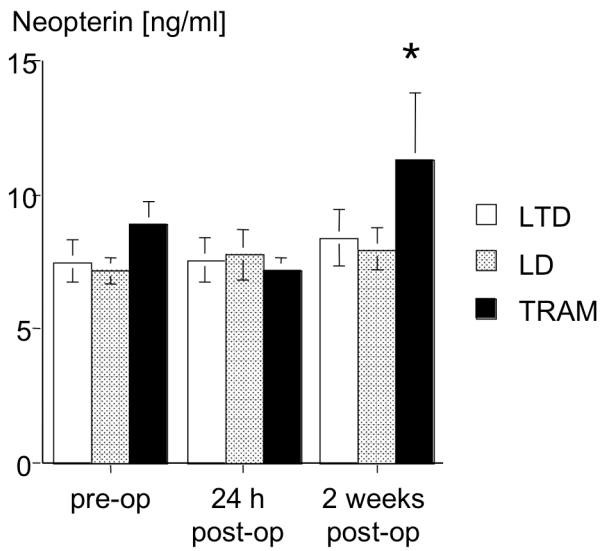
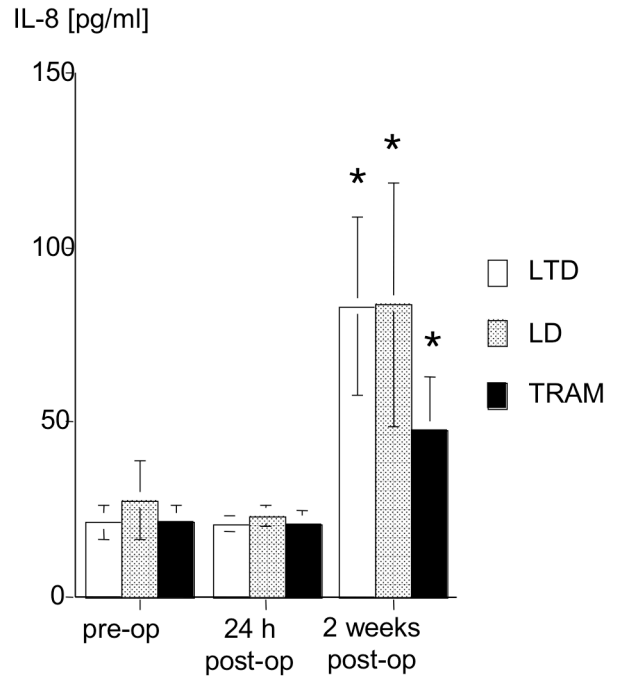
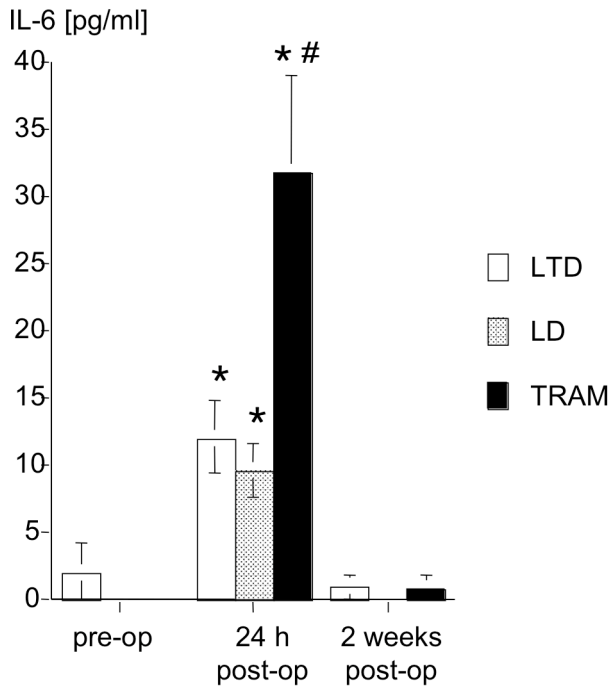


Figure 4: Interleukin-6 levels, IL-8 levels, and neopterin levels preoperatively, 24 hours, and 2 weeks postoperatively. * indicates significant difference to preoperative values in the same group, # indicates significant difference between groups.

Paper II

Demographic data, duration of surgery, anhepatic time and veno-venous bypass time did not differ between groups. Median CIT was 14.8 hours (range 12.5 - 17.7) in group 1 versus 9.3 hours (range 5.7 - 10.5) in group 2.

Plasma levels of the pro-inflammatory mediators are shown in table 2. The concentration of all mediators was significantly elevated in both groups 120 minutes after reperfusion compared to preoperative as well as values obtained 1 minute before reperfusion. In patients receiving a liver with a CIT > 12 h, IL-8 was increased compared to patients receiving a liver with a CIT < 12 h ($p \leq 0.05$).

	preoperative	1 min before reperfusion	120 min after reperfusion
C3a [ng/ml]			
>12 h CIT	263 ± 40	258 ± 84	666 ± 145 *
<12 h CIT	414 ± 75	425 ± 123	752 ± 157 *
SC5b-9 [ng/ml]			
>12 h CIT	300 ± 46	387 ± 99	1373 ± 511 *
<12 h CIT	284 ± 56	283 ± 42	1744 ± 540 *
Neopterin [nmol/ml]			
>12 h CIT	4.7 ± 1.2	4.5 ± 0.9	5.1 ± 1.0 *
<12 h CIT	2.4 ± 0.3	2.2 ± 0.3	3.5 ± 0.4 *
IL-6 [pg/ml]			
>12 h CIT	117 ± 55	130 ± 42	297 ± 60 *
<12 h CIT	26 ± 18	41 ± 11	172 ± 71 *
IL-8 [pg/ml]			
>12 h CIT	238 ± 79	349 ± 94	3750 ± 633 * #
<12 h CIT	277 ± 78	190 ± 31	1433 ± 341 *

Table 2: Plasma levels of complement split products, neopterin, IL-6, and IL-8 in patients undergoing OLT with a CIT of more or less than 12 hours. * indicates statistical significance within the same group, # indicates significant difference between groups; mean ± SEM are given.

Paper III

Groups were comparable regarding body mass index and ASA physical status, but patients undergoing aortic surgery were older than patients undergoing liver surgery (76 years [range 57 -84] vs. 56.5 years [range 33 - 77]). Time from the beginning of blood collection until the start of the washing procedure was significantly shorter in patients undergoing aortic surgery (figure 5).

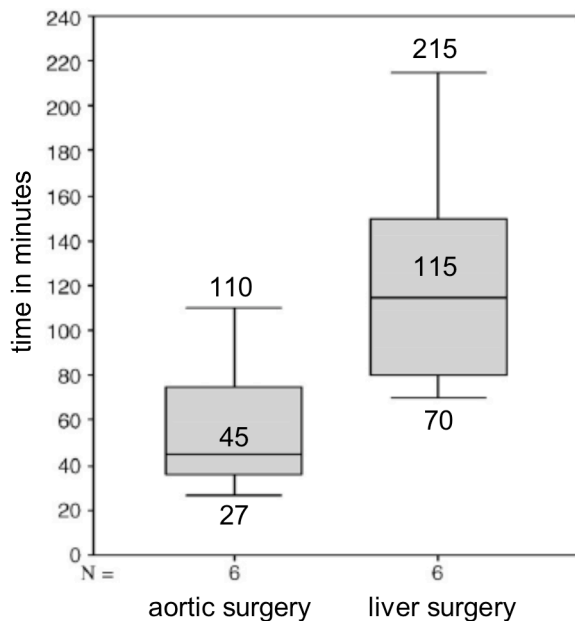


Figure 5: Minutes from blood sampling in the reservoir until the start of the washing procedure; median and ranges are given.

TNF- α was below the detection limit in all samples investigated. In the liver resection group IL-6, C3a, and C5b-9 were significantly lower in the autologous RBC than in patients' blood, in the aortic surgery group this was only observed for C3a. Between groups, plasma levels were significantly different in patients' blood for IL-6 and IL-10 ($p < 0.004$, and < 0.05 , respectively), in the reservoir for IL-10 ($p = 0.013$), and in the autologous RBC for C5b-9 ($p = 0.004$) (figure 6).

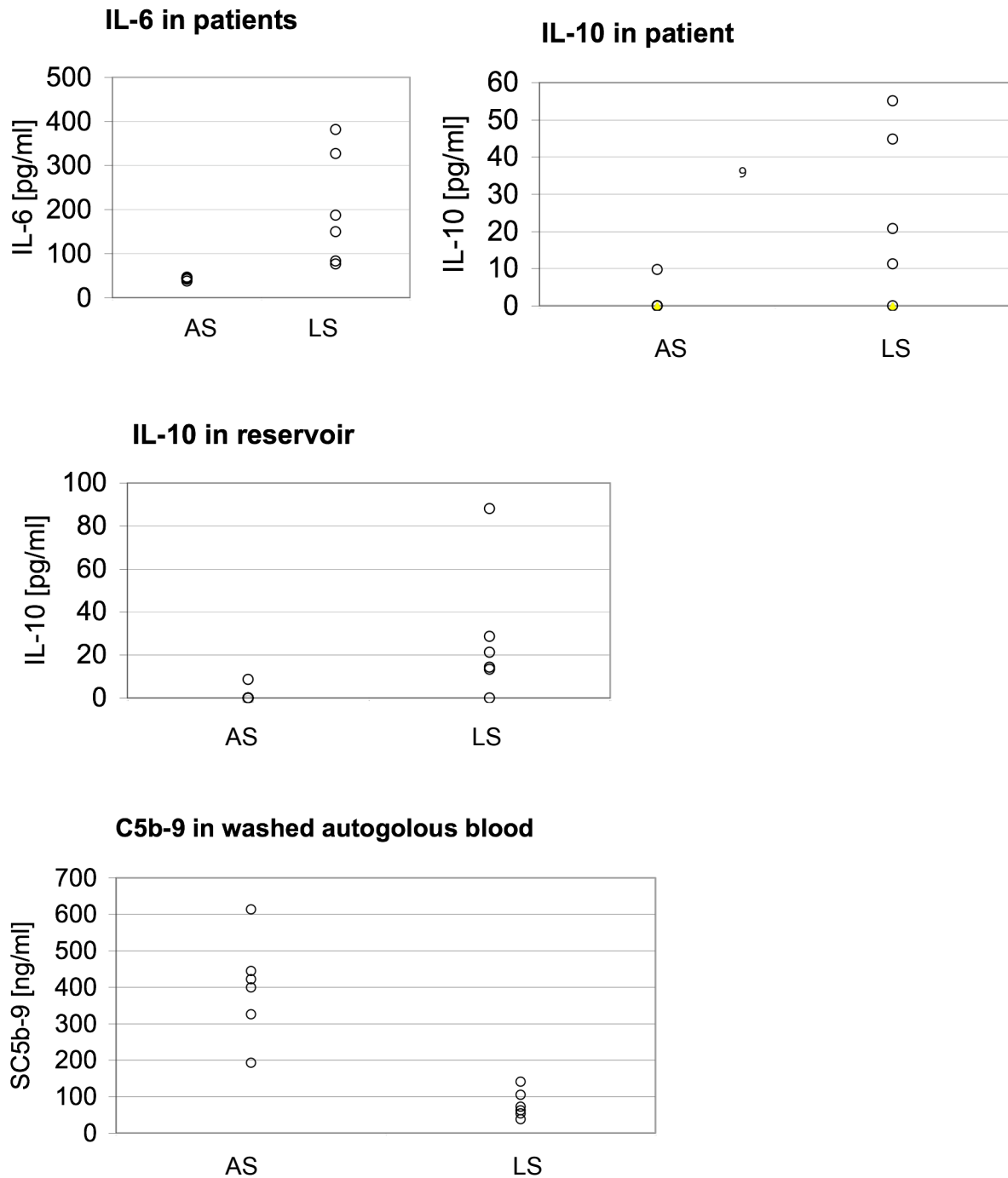


Figure 6: significant differences between patients undergoing aortic surgery (AS) or liver surgery (LS).

Nine of 24 samples showed bacterial growth (table 3). The species isolated and the number of colonies were identical in the reservoir and the processed blood in individual patients. Contamination occurred during blood collection in the reservoir in 4 patients.

The species cultured from all 3 contaminated samples taken from the control group and from 4 samples in the study group were species of the commensal skin microflora isolated in small numbers. However, in the liver resection group contamination with intestinal flora could not be excluded in one patient (liver resection patient 3).

patient no	aortic surgery		liver surgery	
	CS-reservoir	autologous RBC	CS-reservoir	autologous RBC
1	∅	∅	∅	∅
2	∅	∅	∅	∅
3	∅	∅	MC (<10 ² /ml)	MC (<10 ³ /ml)
4	∅	Prop. (a.e.)	CNS (a.e.)	CNS (a.e.)
5	CNS (<10 ³ /ml)	CNS (<10 ³ /ml)	∅	∅
6	∅	∅	CNS (a.e.)	CNS (a.e.)

Table 3: Microbiological results. ∅: no growth; a.e.: after enrichment; CNS: coagulase negative staphylococci; MC: mixed culture; Prop.: Propioni bacteria.

Paper IV

Patients were comparable regarding age. The preoperative hemoglobin concentrations were significantly higher in patients in whom heparin-coated tubing systems were used 136 (130.0 - 143.3) vs 126 (120.8 - 134.3) g/l.

Comparing pro-inflammatory mediators within each group in venous blood to salvaged blood, IL-6, IL-8, C3a, and PMN-elastase were significantly elevated in both groups. For C5b-9 this only applied for blood collected with heparin coated tubing systems (table 4). IL-1 β was below the detection limit, and TNF- α values were within the normal range in all samples investigated.

Levels of pro-inflammatory mediators were not different between blood salvaged with heparin coated versus non-heparin coated tubing systems (table 4).

	preoperative venous blood	intraoperative salvaged blood
C3a [ng/ml]		
heparin coated	138 (138 - 138)	703 (666 - 858) *
non-heparin coated	138 (138 - 189)	726 (451 - 844) *
SC5b-9 [ng/ml]		
heparin coated	124 (90 - 142)	196 (142 - 265) *
non-heparin coated	153 (102 - 187)	138 (73 - 222)
PMN-elastase [ng/ml]		
heparin coated	33 (26 - 39)	364 (298 - 1514) *
non-heparin coated	42 (31 - 52)	1393 (521 - 2770) *
IL-6 [pg/ml]		
heparin coated	0 (0 - 4)	15 (2 - 64) *
non-heparin coated	0 (0 - 4)	11 (4 - 22) *
IL-8 [pg/ml]		
heparin coated	5 (4 - 6)	55 (36 - 86) *
non-heparin coated	4 (3 - 4)	39 (32 - 57) *

Table 4: Plasma concentrations of pro-inflammatory mediators in preoperative, venous versus intraoperative, salvaged blood. In group 1 heparin coated tubing systems were used, in group 2 non-heparin coated ones. * indicates statistical significance between preoperative values and plasma concentrations measured within the same group; median and 25th – 75th percentile are given.

Discussion

Reconstructive surgery

Besides psychological well-being and the cosmetic result obtained, the invasiveness of the procedure should be taken into consideration in plastic surgery, especially, if different treatment alternatives are available. One way to compare surgical stress is to evaluate the inflammatory response, as pro-inflammatory mediators in particular seem to reflect the trauma encountered [101-103].

IL-6 plasma concentrations were elevated 24 hours after surgery in all groups in our study. They were significantly higher in TRAM operated women compared to women with LD and LTD procedures (figure 4). As the TRAM-flap includes the abdominal muscle girdle compared to LD and LTD procedures involving adjacent soft tissue only, these findings may reflect the more extensive tissue trauma encountered during TRAM procedures [104]. In major surgery, however, IL-6 plasma concentrations as high as 500 pg/ml and around 250 pg/ml have been reported 24 hours after liver surgery or uneventful coronary artery bypass surgery, respectively [49, 105, 106]. Compared to these data, IL-6 plasma levels in all three procedures investigated in this study have to be regarded as low. Although LTD, LD, and the TRAM procedure are major operations in the field of plastic surgery, their effect on immunomodulation after the surgical trauma seems to be minor.

This may also explain why TNF- α concentrations were not elevated in our study. However, systemic detection of pro-inflammatory mediators does not necessarily reflect the inflammatory response at the site of trauma. In otherwise healthy patients, perioperative plasma concentrations of TNF- α usually undulate around the detection limit during and after abdominal surgery, but in wound fluid, they have been shown to be significantly elevated. The same applies to IL-6 concentrations: the IL-6 response to injury in peripheral blood is uniquely consistent, peaking the day after surgery and declining thereafter. Depending on the method and time points of wound fluid collection, however, IL-6 concentrations were found to be elevated up to 1000 pg/ml during the operation, and are reported to be as high as 25 000 pg/ml 24 hours after surgery

[107–109]. These differences between wound fluid and peripheral blood have been attributed to a spillover of pro-inflammatory mediators. Entering the systemic circulation, they are diluted and as part of the host defense mechanism cleared from the circulation. If these interactions are unbalanced, circulating pro-inflammatory mediators may affect remote organs, finally resulting in SIRS or sepsis [77, 110].

As part of the innate immune response to trauma, macrophages are drawn to the site of the insult and levels peak after 2 - 4 days, mainly to clear the site of trauma by phagocytosis. Being activated by IFN- γ , a second subpopulation of macrophages appears later in inflammation, mediating tissue repair as part of the adaptive, cellular immune response (figure 7) [111].

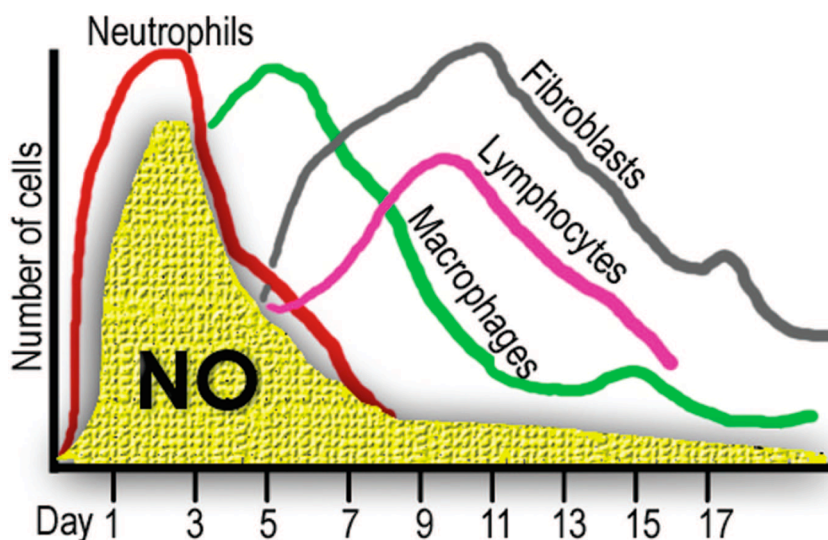


Figure 7: cells involved in wound healing (from: Broughton G 2nd, et al.: The basic science of wound healing. *Plast Reconstr Surg* 2006; 117:12-34.)

Neopterin, a marker associated with activation of cellular immunity, was elevated in TRAM operated women 2 weeks after the procedure only (figure 4). This may reflect the TRAM procedure being the most extensive operation performed in our study, and underline, that macrophage activation for extensive connective tissue repair was still ongoing.

In general, commonly used biomaterial is physically and chemically stable, non-immunogenic, and non-toxic. However, it has been observed that the normal tissue

response to silicone implants involves an inflammatory infiltration, resulting in the formation of a collagenous capsule surrounding the foreign material [112, 113]. It appears that the response to implantation of biomaterial differs from simple wound healing following injury. Macrophages are attracted by biomaterial, they can even be detected months after the implantation [114, 115]. Additionally, it has been shown that biopsies from peri-implant tissue, taken from individuals with silicone related adverse effects express a pro-inflammatory profile of mediators compared to individuals without implant problems [116]. Miro-Mur et al. exposed peripheral blood monocytes from patients with late adverse reactions to silicone implants, from individuals with silicone implants without adverse reaction, and from healthy controls to silicone. These authors describe a significant increase in TNF- α and IL-6 release from monocytes in all groups upon silicone stimulation, but only monocytes from individuals with silicone related adverse reactions presented significantly higher basal concentrations of IL-6 compared to the other groups. In vitro, T-cells from individuals with adverse reactions to silicone were activated, but they showed no memory T-cells and no production of IFN- γ or IL-2 either [117]. These recent studies seem to shed light on the immunobiology underlying the formation of the collagenous capsule, assuming chronic inflammation rather than an antigen mediated disorder. As macrophages play a pivotal role in both, inflammation and tissue repair, it is surprising, that neopterin plasma concentrations were not elevated in women with silicone implants in our study. But the chronic inflammatory response to biomaterial is usually of short duration only and is confined to the implant site [118]. Therefore, we might have missed altered plasma concentrations due to blood sampling time points in our study, and cell mediated immunity, as reflected by neopterin, does not seem to play a major role in the formation of the capsule surrounding silicone implants.

Like TNF- α , and neopterin plasma concentrations, IL-8 levels did not increase 24 hours after the operation in either group. However, 2 weeks after the operation IL-8 plasma concentrations were elevated in all 3 groups, the highest levels being measured in those 2 groups (LTD and LD operated women) requiring saline filled silicone implants for reconstruction (figure 4). IL-8 mainly attracts neutrophils along a gradient to the site of trauma, usually causing neutrophils to peak during the first 24 hours after the insult (figure 7). In parallel to neutrophils, IL-8 plasma concentrations increase within the first day after surgery and gradually decline thereafter. Thus our findings may reflect an ongoing systemic immunomodulation in those women where silicone implants were

used for breast reconstruction. Unfortunately, we did not measure neutrophil elastase or obtain histological samples of the tissue surrounding the implant to support our findings. It has also been suggested that increased plasma levels of IL-8 may exert anti-inflammatory effects: as neutrophils are recruited along a gradient to the site of inflammation, elevated IL-8 concentrations are rather drawing cells away than attracting them to the site of inflammation [119].

Cold ischemia time

With reperfusion of the grafted liver high plasma levels of pro-inflammatory mediators have been detected in the systemic circulation. They result from contact activation from the veno-venous bypass circuit, and intestinal and lower extremity ischemia during the anhepatic phase, but are primarily released from the liver graft itself upon reperfusion. This preservation injury mainly affects sinusoidal endothelial cells [19]. Within minutes after reperfusion, Kupffer cells are activated [120], releasing an array of pro-inflammatory mediators including cytokines like TNF- α , IL-1, and chemokines, as well as recruiting neutrophils to the liver [121]. Pesonen et al. have shown that hepatic sequestration and intrahepatic activation of neutrophils occurs with reperfusion in human OLT [20]. Moreover, Kataoka et al. demonstrated that duration of cold ischemia time correlates to intrahepatic PMN recruitment and activation after reperfusion. They used an animal model, comparing rat livers exposed to a CIT of 24 hours compared to a CIT of 3 and 6 hours, respectively, and evaluated gene expression and histology in the grafted liver [122]. It has also been suggested that a short CIT may protect against severe neutrophil mediated hepatic injury despite extensive neutrophil activation [123], the underlying mechanism of the observation is not yet clear.

As Kupffer cells, resident liver macrophages, are the major players in the above described early ischemia / reperfusion (I/R) injury in liver transplantation, it is surprising that neopterin plasma concentrations were within the normal range at all time points investigated in our study. Muller and coworkers reported neopterin to be released from the grafted liver, but values returned to baseline within 2 hours after reperfusion [21]. Tomasdottir et al. found neopterin plasma levels to be significantly elevated for up to 6 hours after reperfusion, but the values measured were within the same range as plasma

concentrations in our study [124]. It seems likely that Kupffer cell activation early after reperfusion does not translate into systemic release of neopterin, but exerts its action mainly locally in an autocrine or paracrine manner.

By contrast, pro-inflammatory cytokines are released into the systemic circulation upon reperfusion. Wanner and coworkers described an early increase of TNF- α and IL-1 α within minutes after reperfusion, while an elevation of IL-6 concentration was observed with a delay of 2 hours [125]. Other groups, also obtaining blood samples prior to reperfusion, found a steady increase in IL-6 plasma levels as early as during the anhepatic phase [15, 126], but observed peak plasma concentrations of IL-6 between 60 and 120 minutes after reperfusion, only.

In our study, IL-6 plasma levels increased only slightly prior to reperfusion, but were significantly elevated 120 minutes after reperfusion in both groups (table 2). One possible source of IL-6 secretion are Kupffer cells, being stimulated by endotoxin, IL-1 and TNF- α after cold preservation. In vitro, administration of dexamethasone prior to liver injury was able to almost completely inhibit the release of IL-6 from activated Kupffer cells [127, 128]. As dexamethasone was administered with induction of anesthesia in our study, this could account for the only moderate elevation of IL-6 observed in our patients compared to other studies in human subjects. Another source of pro-inflammatory cytokines involved in liver I/R injury are neutrophils. Pesonen and coworkers have shown that neutrophil activation during CIT does not correlate to IL-6 concentrations released upon reperfusion [20]. Measuring cytokine release in the preservation solution effluent at the moment of reperfusion, Gerlach et al. found neither IL-6 nor TNF- α plasma concentrations to be elevated compared to donor plasma concentration, nor to be correlated to the duration of CIT [129]. They concluded that University of Wisconsin solution successfully inhibits liberation of the investigated cytokines during cold preservation. Taking together, these results may explain why we did not find any difference in IL-6 plasma concentrations between groups.

Interleukin-8, in contrast, is released from the grafted liver upon reperfusion, as shown by blood sampled simultaneously from the portal vein and from the hepatic vein [20]. IL-8 concentrations correlated to neutrophil sequestration and activation measured at the same time. In our study, IL-8 concentrations increased to a mean of 3750 ± 633

versus 1433 ± 341 pg/ml 120 minutes after reperfusion in patients with a CIT of > 12 hours or a CIT < 12 hours, respectively. The result was statistically significant between groups. Mueller and colleagues observed plasma levels of IL-8 in the same range as our group. They closely monitored patients' cytokine concentrations for 120 hours post transplantation and correlated them to early graft function. In patients with good early graft function, IL-8 decreased rapidly within 24 hours, whereas in patients with moderate or poor early graft function, significantly elevated plasma concentrations were detected throughout the study period [21]. However, they could not establish any correlation between IL-6 plasma levels and early graft function. It was only recently, that the role of chemokines like IL-8 in liver injury and recovery from I/R injury was described in more detail: besides induction of chemotaxis and respiratory burst on neutrophils, facilitating angiogenesis in binding to endothelial cells [130], they may also induce proliferation of hepatocytes after hepatectomy. This requires a moderate increase of chemokines only [131]. Kuboti et al. showed that large increases of chemokines, as occurs after I/R injury, might be hepatotoxic and oppose hepatocyte proliferation and regeneration [132]. High IL-8 plasma concentrations in patients receiving a liver with a CIT > 12 hours in our study may reflect the cytotoxic effects of chemokines, but unfortunately we did not follow up our patients any further.

The complement cascade can rapidly be activated during early reperfusion, resulting in Kupffer cell activation as well as neutrophil recruitment into sinusoids [121, 133]. In addition to this pro-inflammatory effect, the membrane attack complex can directly cause cell injury [134] and seems to target on hepatocytes mainly [135]. Both, anaphylatoxins and the membrane attack complex, have been shown to be elevated during OLT [136]. This is in line with findings in our study. However, the duration of CIT did not influence the systemic release of complement split products 2 hours after reperfusion.

Salvaged shed blood

Pro-inflammatory mediators are known to be elevated in collected shed blood, regardless of the operation performed. This may be explained by the observation that inflammatory mediators are released within minutes at the site of injury to restore tissue

integrity. They reflect the degree of tissue trauma encountered, have been shown to differ between acute and chronic wounds, and have been reported to predict outcome after wound healing [137, 138].

In our study, comparing salvaged blood collected during liver surgery to blood collected during aortic surgery, pro-inflammatory mediators were elevated in shed blood collected in the CS-reservoir in both groups. Although individual concentrations in the reservoir were higher in patients undergoing liver resection, cell separation and the washing procedure reduced complement split products and cytokines to levels accepted in aortic surgery. Additionally, in the liver resection group concentrations of IL-6, C3a, and C5b-9 were significantly lower in the autologous RBC compared to venous blood of the patients. In healthy subjects, however, plasma levels have been found to be even lower than in the RBCs in our study [139].

At the time of cell processing, IL-6 plasma concentrations were already significantly higher in patients undergoing liver resection compared to the control group. This may be attributed to the duration of the collection time in the reservoir: 45 minutes in the aortic surgery group compared to 110 minutes in the liver resection group, respectively. Bentzien and coworkers investigated shed blood in 100 patients undergoing knee replacement surgery, and found a positive correlation between IL-6 concentrations in the shed blood and the time when samples were taken from the reservoir [140]. They could not establish such a correlation with any other mediator investigated.

The longer collection time might also account for the elevated IL-10 levels in the liver resection group, in the blood of the patients as well as in the reservoir. IL-10 release is induced by pro-inflammatory cytokines and appears later in the inflammatory response to trauma [87]. Van der Poll and colleagues investigated the time frame of IL-10 synthesis after an inflammatory insult. They challenged healthy volunteers with TNF- α and observed that IL-10 peaked 45 minutes after TNF- α administration [141].

It has previously been shown that salvaged shed blood in orthopedic as well as in cardiac surgery contains high concentrations of complement split products [106, 142]. Contact of circulating blood with foreign material may account for the activation of the complement cascade [143]. Additionally, bile from dissected bile ducts or bacterial

contamination of the collected blood could activate the complement cascade during liver resection. However, the washing procedure in the CS reduced C3a and C5b-9 levels below patients' plasma concentrations in the liver resection group. In the aortic surgery group, C5b-9 was reduced in the autologous RBC compared to the reservoir, but exceeded patients' plasma concentrations 2.5 times. Apart from the washing volume used (500 ml in aortic surgery and 1000 ml in liver surgery, respectively), the CS settings were identical in both groups. Investigating different autotransfusion devices, Tylman and coworkers found that the Haemonetics Cell Saver 5[®] reduced elevated concentrations of C3a and C5b-9 effectively when using a washing volume of 1000 ml per cycle [144].

Worrying about the release of inflammatory mediators, and about the promotion of infection, SIRS or even sepsis upon transfusion, blood collection from infectious sites is deemed a contraindication so far. Special filters seem to reduce the bacterial load only, and therefore they are not considered to be safe enough to be introduced into clinical practice yet [145, 146]. However, bacterial contamination of the salvaged blood is a known - and accepted - phenomenon. In so-called "sterile" operations in orthopedic and neurosurgery, 28 % up to 39 % of the samples obtained from intraoperatively salvaged autologous RBCs were contaminated [39, 147, 148]. Ezzedine and coworkers [38] and Sugai et al. [39] investigated the samples further and isolated skin and environmental contaminants in the cultures, respectively. They found no correlation between bacteriologic results and complications or laboratory findings indicating infectious disease afterwards. In our study, 42 % of the cultures obtained from the autologous RBC were bacteriologically contaminated. In 4 of the 5 samples coagulase-negative staphylococci and propioni bacteria were isolated in small numbers, both being members of the commensal skin microflora. This is in agreement with the above mentioned studies. But in one patient undergoing liver resection, we could not exclude contamination with intestinal flora, e.g. coli bacteria. Although we did not obtain blood cultures from patients, we assume that the contamination occurred during blood salvage, either due to retrograde contamination from bile ducts or due to iatrogenic factors during the sampling and washing process. The patient did not show any signs of infection perioperatively, nor was the gut lumen perforated or opened during the procedure. This finding deserves further investigation and critical evaluation before considering transfusion of autologous RBC in liver resection.

In operations with a moderate intraoperative blood loss only, “washed” autotransfusion systems like the CS are commonly not used. However, especially in orthopedic surgery, postoperative salvage and filtration of wound fluid is an established method to reduce the need for allogeneic blood transfusion [48, 138]. It is known that postoperatively salvaged blood that is not centrifuged and washed contains high concentrations of various inflammatory mediators. This has been shown to be without any clinical effect, if volumes less than 1500 ml were transfused [47, 48, 149]. Nevertheless, it is still discussed controversially whether washed or unwashed shed blood should be transfused postoperatively [150, 151].

Complement activation plays an important role in the inflammatory response to extracorporeal circulation in cardiac surgery [152, 153]. The impact of different tubing systems including heparin coating has been studied and discussed extensively in this setting. Most studies however, investigating transfusion of postoperatively filtered wound blood in orthopedic surgery focus on clinical parameters to assess the quality of the blood product only. Few studies investigated inflammatory mediators including complement split products. In two studies, C3a levels were reported to be as high as 3956 ng/ml [154] or 4210 ng/ml [155] in postoperatively salvaged shed blood, respectively. Upon transfusion neither systemic complement activation nor clinical complications were observed in the otherwise healthy patients. Both tubing systems tested in our study activated the complement cascade, but C3a concentrations in the salvaged shed blood (703 ng/ml and 726 ng/ml, respectively) were lower than those reported in the above mentioned studies during postoperative blood collection.

We found similar results for pro-inflammatory cytokines investigated. Both, IL-6 and IL-8 concentrations were elevated in the collected blood, but the concentrations in our study were lower than previously reported in postoperative salvage [43, 142, 156]. The major difference between our study and postoperatively collected shed blood is the time elapsed from starting the blood collection until finally transfusing the salvaged blood. Intraoperatively, transfusion is started as soon as possible, whereas postoperatively, blood is usually collected for 6 hours, the maximal time regarded as safe and approved for salvage of shed blood [157]. This exposes the collected blood to tissue factors from the surgical site, foreign material, air, and activated leukocytes for a prolonged period of

time. These factors may contribute to the activation of plasma cascades and cellular systems, affecting the quality of the salvaged blood.

Exposing blood to artificial surfaces promotes a systemic inflammatory response including activation of leukocytes, platelets, and the complement cascade, and it induces the synthesis and release of mainly pro-inflammatory cytokines. Lappegård and colleagues exposed whole blood to polyvinylchloride. In their setting, chemokines, including IL-8, and growth-factors were induced by polyvinylchloride, but traditional pro-inflammatory cytokines like IL-1 β , IL-6, and IL-10 were virtually unaffected. Inhibition of complement activation reduced the formation of almost all mediators significantly, whereas heparin coating of the surface abolished the entire inflammatory response [158, 159]. However, contributing factors like suction or roller pumps, mediators released from the site of surgery or endothelium were not considered in this model. This may explain the conflicting results of heparin coating of extracorporeal perfusion systems during cardio-pulmonary bypass regarding the release and activation of inflammatory mediators [49, 160]. We did not find any difference in pro-inflammatory cytokines or complement split products between the blood salvaged with heparin-coated surfaces or with non-heparin coated ones. The same applies to PMN-elastase, although again, laboratory studies suggested that its activity is inhibited by heparin [161].

Conclusion

1. Flap procedures, involving connective tissue for breast reconstruction, stimulate a pro-inflammatory response; regarding immunomodulation, they should be considered as minor procedures.

The use of silicone implants seems to modulate the immune response.

2. Pro-inflammatory cytokines and complement split products are released upon reperfusion in OLT, but only the chemokine IL-8 correlates to the duration of CIT.
3. In salvaged shed blood, inflammatory mediators are higher in liver resection than in vascular surgery. Cell separation and the washing procedures reduce inflammatory mediators to levels commonly accepted in aortic surgery.

Contamination of shed blood with intestinal flora cannot be excluded in liver surgery.

4. Blood salvaged intraoperatively during hip arthroplasty contains elevated levels of pro-inflammatory cytokines and complement split products.
5. Heparin-coating of the tubing systems does not influence the formation of inflammatory mediators.

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Sammanfattning på svenska

Kirurgi leder till ett inflammatoriskt svar som motsvarar omfattningen av ingreppet och detta påverkar resultatet av ingreppet. Syftet med studierna var att studera faktorer som påverkar det inflammatoriska svaret och påverkan av användande av patientens eget blod under operation.

Material och metoder

I fyra prospektiva studier, undersöktes inflammatoriska metaboliter i samband med kirurgi. I: Det inflammatoriska svaret i samband med 3 olika typer av bröstrekonstruktion som skiljer sig i komplexitet och användandet av silikonimplantat i annars identiska patientgrupper analyserades. II: I 18 patienter som genomgick levertransplantation studerades frisättning av pro-inflammatoriska mediatorer och detta ställdes i relation till den transplanterades leverns ischemitid. Mer än 12 timmars ischemitid jämfördes med mindre än 12 timmars ischemitid. III: Med syfte att minska användningen av bankblod i samband med leverkirurgi, studerades kvaliteten på uppsamlat blod. Effekten av tvättning av blod studerades. Resultaten jämfördes med blod uppsamlat och processat under aortakirurgi. IV: I 40 patienter som opererades med insättning av höftprotes studerades pro-inflammatoriska metaboliter i uppsamlat och enbart filtrerat blod. Effekten på pro-inflammatoriska metaboliter av heparinisering av ytorna i uppsamlingsenheten jämfördes med icke hepariniserade ytor.

Resultat

I: IL-6 var förhöjt i alla tre studerade grupperna dag 1 efter operation. IL-8 var signifikant förhöjt 2 veckor efter operation i alla grupperna. Kvinnor med silikonimplantat hade högsta nivåerna av IL-8. II: Plasma koncentrationer av C3a, C5b-9, neopterin, IL-6 och IL-8 var förhöjda 120 minuter efter reperfusion i båda grupperna, och endast IL-8 skiljde sig mellan grupperna. III: Inflammatoriska mediatorer var förhöjda i uppsamlat blod i båda de studerade grupperna. Efter tvättning var IL-6, C3a och C5b-9 lägre i det uppsamlade blodet än i cirkulerande blod i lever resektionsgruppen. Kontamination med

tarmflora kunde inte exkluderas i en patient. IV: C3a, sC5b-9, PMN elastase, IL-6 och IL-8 var förhöjt i uppsamlat blod i båda grupperna utan att det var någon skillnad mellan om ytorna var hepariniserade eller ej.

Konklusioner

Bröstrekonstruktion med flap metod leder till ett mindre inflammatoriskt svar. Metoden med silikonimplantation förefaller ha en immunmodulerande effekt. Pro-inflammatoriska mediatorer frisätts under reperfusion av transplanterad lever. Endast frisättning av IL-8 korrelerar positivt till längden av ischemitid. Uppsamlat blod i samband med leverresektion innehåller höga nivåer av inflammatoriska mediatorer. Efter tvättning och centrifugering förefaller det vara lika säkert att återtransfundera det uppsamlade blodet som i samband med aortakirurgi. Blod som uppsamlas i samband med höftproteskirurgi innehåller höga nivåer av inflammatoriska mediatorer. Heparin behandling av ytorna i uppsamlingssystemen påverkade inte nivåerna av inflammatoriska substanser.