Impact of open abdomen and Vacuum Assisted Closure Device in surgical abdominal sepsis

by

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Abstract

Introduction: Surgical abdominal sepsis has traditionally been managed with by a single staged procedure, otherwise known as primary abdominal closure (PAC). An on demand laparotomy may be performed for post-operative clinical deterioration. Open abdomen and a planned re-laparotomy with vacuum assisted closure (VAC) is an alternate method to single staged procedure. Inflammatory cytokines can potentially help stratify severity of sepsis and guide surgical management. The objective of the study was to identify if inflammatory cytokines could differentiate between PAC and VAC. Secondary objectives were to see if cytokines could predict mortality and characterize longitudinal cytokine profiles during open abdomen management. Severity of disease between surgical groups was compared using the Acute Physiology and Chronic Health Assessment (APACHE)-IV predictive mortality rate (PMR) calculator.

Methods: Prospective case series between December 2011 to June 2013. Patients were included if they met criteria of severe abdominal sepsis/septic shock requiring urgent source control laparotomy (SCL). Blood and peritoneal samples were obtained pre- and post-operatively at primary SCL in patients who underwent PAC and VAC management. Peritoneal fluid (PF) samples were obtained once the peritoneum was entered. Blood and peritoneal samples were obtained for re-look laparotomies in the VAC group. Samples were centrifuged within 1 hour and stored at -70 degrees Celsius. Samples were analyzed with a Human Cytokine 30-plex Panel and concentrations were reported as pg/ml.

Results: 12 patients were included (4 PAC and 8 VAC). PF cytokine concentrations of IL 6, IL-17, IL-5 and HGF were significantly higher in VAC compared to PAC. Peritoneal fluid at primary SCL did not differentiate between survivors and non-survivors. Pre-operative serum RANTES was significantly elevated in survivors compared to non-survivors. Pre operative serum VEGF, IL-1b, FGF-b, IL-5, IL-4, IL-7 and post-operative serum VEGF, IL-7 differentiated between VAC survivors and non-survivors at second SCL.

Conclusion: VAC management was utilized in patients with elevated peritoneal cytokines compared to single staged procedures. Increased peritoneal inflammatory cytokine concentrations in VAC represent a more severe degree of local sepsis. Pro and anti-inflammatory cytokines are both elevated in the early and late phases of surgical abdominal sepsis.

Preface

All of the work presented was conducted at Vancouver General Hospital (VGH), Jack Bell Research Center, and BC Cancer Research Center at the University of British Columbia (UBC).

"Impact of negative pressure systems on serum and peritoneal cytokines in surgical abdominal sepsis" received approval from the University of British Columbia Research Ethics Board (certificate #H11-02485). I was the lead investigator responsible for original concept design, obtaining patient consent, acquiring, processing and storing test samples. I performed laboratory work along with the guidance of a Life Technologies representative for the Human cytokine 30-plex luminex kit. I performed statistical analysis.

Dr. Andrzej Buczkowski was the lead supervisor, helped develop original concept design, contributed throughout the study and helped with manuscript edits.

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Table of Contents

List of Tables

List of Figures

List of abbreviations

ACS: Abdominal compartment syndrome
CARS: Compensatory anti-inflammatory response syndrome
CMV: Cytomegalovirus
DCS: Damage control laparotomy
EGF: Epidermal growth factor
FGF-b: Fibroblastic growth factor: basic
G-CSF: Granulocyte-colony stimulating factor
GM-CSF: Granulocyte macrophage-colony stimulating factor
HGF: Hepatocyte growth factor
HSV: Herpes simplex virus
ICU: Intensive care unit
IL-1: Interleukin-1b
IL-2: Interleukin-2
IL-2R: Interleukin-2 receptor
IL-3: Interleukin-3
IL-4: Interluekin-4
IL-5: Interleukin-5
IL-6: Interleukin 6
IL-7: Interleukin 7
IL-8: Interleukin 8
IL-10: Interleukin 10
IL-12: Interleukin 12
IL-13: Interleukin 13
IL-15: Interleukin 15
IL-17: Interleukin 17
IFN-a: Interferon alpha
IFN-y: Interferon gamma
IP-10: Interferon inducible protein-10
LPS: Lipopolysaccharide
MCP-1: Monocyte chemo attractant protein-1
MIG: Monokine induced by gamma interferon
MIP-1a: Macrophage inflammatory protein 1a
MIP-1b: Macrophage inflammatory protein 1b
NF-kB: Nuclear factor kappa betta
NPWT: Negative pressure wound therapy
PAC: Primary abdominal closure
RANTES: Regulated on activation, normal T cell expressed and secreted
RCT: Randomized control trial
SIRS: Systemic inflammatory response syndrome
SCL: Source control laparotomy
STAT: Signal transducer and activator of transcription
TAC: Temporary abdominal closure
UBC: University of British Columbia
VAC: Vacuum assisted closure

VEGF: Vascular endothelial growth factor
VGH: Vancouver General Hospital

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“When you want to succeed as bad as you want to breath, then you will be successful”

-Eric Thomas

Chapter 1: Introduction

Sepsis is a major cause of morbidity and mortality in critically ill patients and is the 10th leading cause of death in the United States (1). It is the leading cause of mortality in non-cardiac ICUs (2) with mortality rates ranging from 30-50% (3, 4). Mortality rates remain extremely high for abdominal sepsis in ICU patients and surgery often provides the only chance for definitive treatment. In the United States, on average 750,000 people per year are diagnosed with sepsis (5). It is an economic burden that cost the US $20 billion dollars in 2011, and the cost will only increase with a growing incidence of sepsis (6).

Sepsis is the result of a systemic inflammatory response syndrome (SIRS) mediated by the immune system secondary to stimulation from invading pathogens. SIRS is composed of four parameters: temperature <36.0° or >38.0° Celsius, white blood cell count (WBC) <4 or >12 10^3/uL, heart rate >90 bpm, respiratory rate >20/min, or PaC02 <32 (7). SIRS can be due to; trauma, burns, inflammation or non-infectious pancreatitis. Sepsis has traditionally been defined by the presence of ≥ 2 components of SIRS and a suspected or confirmed source of infection (8, 9).

Sepsis, severe sepsis and septic shock represent three stages within the spectrum of sepsis. Sepsis may present clinically with signs of SIRS criteria or other non-specific symptoms. Severe sepsis results from organ hypo-perfusion and may lead to multi-organ failure often represented by acute lung and kidney injury. Shock liver, delirium, dilated cardiomyopathy or disseminated intravascular coagulation (DIC) and hypo-perfusion are examples of organ dysfunction that may occur. Septic shock is a form of distributive shock in which the cardiovascular system is unable to maintain blood pressure despite

appropriate fluid resuscitation. Severe sepsis and septic shock can be thought of as different syndromes, because not all patients with severe sepsis will develop septic shock and vice versa (10).

The mainstay of sepsis treatment involves early administration of fluids (11), antibiotics (12) and achieving source control (13). River's protocol (14) was a milestone for reduction of sepsis mortality and morbidity by introducing the concept of early goal directed therapy in a bundled approach. Identified septic patients were hemodynamically monitored and resuscitated according to pre-determined targets of central venous pressure, mean arterial pressure and mixed venous oxygen saturation. River's protocol provided a platform for the advancement of sepsis management that has continued to evolve (15). Goal directed therapy has also shown to have a reduction in mortality in high-risk surgical patients (16). Source control involves repairing/removing and treating the infectious source. In abdominal sepsis, source control includes combinations of antibiotics, radiological guided drain placement, or surgery (17-20).

1.1 Pathophysiology

The clinical symptoms of severe sepsis/septic shock are due to the host's immune response, which attempts to locally control invading pathogens via an inflammatory response that simultaneously causes secondary tissue damage (21). Invading pathogens interact with immune cells activating the innate and adaptive immune systems, complement, coagulation, and autonomic nervous systems (22, 23). The result is an excessive production and imbalance of inflammatory mediators, cytokines and chemokines.

Cytokines are small-secreted proteins that are released by cells of the innate and adaptive immune systems and communicate in an autocrine and paracrine fashion (24). Chemokines are composed of groups of proteins that are primarily responsible for cell recruitment. The molecular interactions between pathogens, immune cells and cytokine signaling is what drives the early/late clinical manifestations of sepsis, severe sepsis and septic shock. A relatively small infection may induce an over the top pro-inflammatory response or a large pathogenic insult may cause an extremely high but necessary rise in pro-inflammatory cytokines (25). The degree of inflammatory response is also dependent on age, medical co-morbidities, immune status and genetic pre-disposition (26, 27).

The immune system is composed of innate and adaptive systems that respond to invading infection. When bacteria, viruses or fungi invade the body they release various molecules including but not limited to; lipopolysaccharide (LPS), peptidoglycan, proteins, and RNA that are collectively called pathogen associated molecular proteins (PAMPs) (28). Damaged and necrotic tissues passively release proteins and other endogenous molecules called damage associated molecular patterns (DAMPs) (29). DAMPs induce sterile inflammation, however immune cells can also actively secrete DAMPs secondary to sepsis, which contribute to patient mortality (30). Signaling pathways that are activated secondary to damaged or necrotic tissues are able to induce pro-inflammatory cytokine responses similar to PAMPs (31) highlighting the synergistic effect of inflammation (32). The innate immune system is the first line of defense and its role is to quickly identify and respond to PAMPs and DAMPs. Epithelial barriers, neutrophils, monocytes, macrophages, natural killer (NK) cells and dendritic cells are all part of the innate immune system. Neutrophils are recruited from the vascular system to

sites of infection through chemokines and are one of the first cells to respond to infection through the release of several inflammatory mediators.

In response to; microbial products, TNF-a, and other cytokines, neutrophils release chemokines, pro-/anti-inflammatory cytokines, colony-stimulating and angiogenic factors (33). More specifically, neutrophils release the pro-inflammatory cytokines IL-6, IL-8 and IL-17, which augment the inflammatory response and send chemotactic signals for cell recruitment (34). Additionally, they produce free oxygen radicals, stimulate endothelial damage, form neutrophil extracellular traps (NETs) and kill bacteria through phagocytosis (35). Neutrophils form NETs in response to pathogens via the release of nuclear components and anti-microbial proteins, which coat and trap pathogenic bacteria so that they are immobilized (36). Anti-microbial proteins immobilize pathogens acting as markers for neutrophils to engulf and phagocytose bacteria. This killing mechanism is referred to as NETosis and is independent from apoptosis and cell necrosis (37). NETs have been shown to be beneficial in eradicating bacteria. However, excessive neutrophil activation may worsen the inflammatory process (38) by causing significant tissue damage and alterations in microvascular perfusion, leading to thrombosis and multi-organ dysfunction (39). Activated neutrophils play a protective role in sepsis, however excessive stimulation and activation of neutrophils contributes to the development of severe sepsis (40).

Macrophages are present in all tissues and are the major immune cell type involved in cytokine release and cell interaction. They can be expressed as M1 or M2 subtypes depending on exposure to cytokine signaling (41). Current understanding is that M1 macrophages promote the initial pro-inflammatory response in disease and M2

macrophages modulate wound repair, angiogenesis and immunosuppression (42). IFN-y and TNF-a promote activation into M1 macrophages resulting in the release of high levels of pro-inflammatory cytokines IL-1, IL-6, IL-23 and low levels of IL-10 (43). M2 macrophages carry out various functions mediated by different subtypes and are stimulated by cytokines IL-4, IL-10 and IL-13 (44). Despite this classification of M1 and M2 macrophages, there is a spectrum of cytokines that are released by a hybrid of macrophage phenotypes dependent on paracrine cytokine signaling (45).

Monocytes, macrophages and dendritic cells are a specialized group of antigen presenting cells (APCs) with pattern recognition receptors (PRRs) located on the cell surface. Recognition of PAMPs or DAMPs by PRRs on APCs induces downstream signaling cascades leading to transcription of inflammatory cytokines (46). Importantly, APCs provide a bridge of communication between the innate and adaptive immune systems. APCs express major histocompatibility complexes (MHCs) that bind to special peptide fragments that have been processed by PRRs and are recognized by T lymphocytes (47). APCs process internalized bacterial products via MHC-II complexes that interact with CD4 T lymphocytes (48). Endogenous or viral proteins are bound to MHC-I complexes and interact with CD8 T lymphocytes (49).

PRRs are a group of trans-membrane and intra-cellular receptors with families of different receptor subtypes responsible for recognizing exogenous pathogens (PAMPs) and endogenous stimuli (DAMPS). PRRs are located on monocytes, macrophages, dendritic cells, NK cells, neutrophils, endothelial cells, and epithelial cells of the gastro-intestinal, respiratory and endothelial systems (50). The main groups of PRRs are: toll like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NOD),

inflammasomes and retinoic acid-inducible gene-1-like (RIG-like) receptors (28). TLRs and NOD-like receptors have most commonly been studied in sepsis. They are located on cells of the innate immune system and are able to induce phagocytosis, inflammation and APC maturation (51). Different pathways of signal transduction for both TLRs and NOD-like receptors are able to activate nuclear factor kB (NF-kB) and lead to the development of SIRS (52). Inactivated NF-kB is transferred from the cytosol to the nucleus where it becomes activated and regulates the immune system through transcription of inflammatory cytokines (53).

NOD1 and NOD2 receptors are intra-cellular PPRs that recognize gram positive/negative bacterial PAMPs and are the main NOD receptors found in sepsis (54). TLRs are a large family of mostly trans-membrane PRRs that are predominately located on macrophages, dendritic, NK and endothelial cells (55). The intracellular portion of TLRs share similar homology with IL-1R receptor and this amplifies transcription of pro-inflammatory cytokines when NF-kB is activated (56). Full review of TLR activation and required accessory molecules can be found here (57, 58). Additionally, activation of TLRs on endothelial cells results in the release IL-6 and IL-8 thereby promoting endothelial coagulopathy, microvascular thrombosis and vascular leak (59).

The classic example of gram-negative bacteria induced sepsis involves the release of the endotoxin lipopolysaccharide (LPS) and its interaction with TLR4 (60). APCs are able to recognize and bind to LPS via LPS binding protein. The initial binding of LPS to the surface of activated macrophages causes a pro-inflammatory cytokine release of macrophage inhibitory factor (MIF), which does not require transcriptional activation and promotes TNF-a release (61). LPS binds to CD14, an accessory-binding molecule located

on the surface of macrophages and helps transport LPS to TLR4. The interaction of LPS and TLR4 results in the formation of a LPS-TLR4 complex, which is enhanced by MD2 and is dependent on MyD88 proteins. MyD88 is a signaling protein that aids the LPS-TLR4 complex in signal transduction and activation of NF-kB (62). NF-kB is transported from the cytoplasm into the nucleus where transcription of pro-inflammatory cytokines (TNF-a, interleukin IL-1, IL-2, IL-6, IL-8, IL-12) and IL-10 occurs. In LPS induced activation of TLR4, TNF-a and its receptor play a primary role of initiating the pro-inflammatory response. Because IL-1R shares similar homology with TLRs, TNF-a activates IL-1 to bind to its IL-1R receptor inducing signaling cascades that amplify pro-inflammatory gene expression (63). In addition to promoting expression of pro-inflammatory cytokines, the LPS-PRR interaction induces simultaneous counter-regulatory mechanisms in an attempt to control the pro-inflammatory cascade (64).

Traditionally, severe sepsis and septic shock were thought of as a syndrome that was composed of a single phase of pro-inflammatory mediators, which contributed to morbidity and mortality (53). This concept led to great pharmaceutical interest in finding a means to inhibit the pro-inflammatory cascade in an attempt to reduce mortality. Over 20 studies in 2 decades, each have each failed to significantly improve sepsis outcomes (65-67). These failed clinical trials highlight the fact that pro-inflammatory cascades induced by the immune system are activated via many different pathways and that single inhibitory agents are insufficient to significantly reduce morbidity and mortality associated with sepsis.

Over time, increasing numbers of critically ill septic patients began to survive the early pro-inflammatory phase due advancements in sepsis treatment, which included:

antibiotics, ICU care and source control. After patients survived the initial pro-inflammatory cytokine storm represented by SIRS, a second phase of sepsis called the, "compensatory anti-inflammatory response syndrome" (CARS) was thought to be entered (68). The CARS phase is a phase of immune paralysis. After surviving the initial septic event, morbidity and mortality are the result of increased susceptibility and resistance of secondary infections. Secondary infections develop because the immune system is unable to mount proper secondary pro-inflammatory responses.

Macrophages have a reduced ability to present bacterial components via the MHC-II complex to T cell lymphocytes, and leukocytes are unable to stimulate appropriate secondary pro-inflammatory cytokines in response to infection (69). Mouse models of abdominal sepsis have shown a reduction in total splenic cells, total lymphocyte counts and IgM expressing B cells 2 days after infection (70). Animals that survive to 9 or 20 days post-induced peritonitis have minimal TNF-a responsiveness to LPS; and have recurrent bacterial infections after the primary septic insult (71). Human studies have provided similar evidence. Patients with severe sepsis who have passed away in the ICU had suppressed immune systems with decreased total splenic lymphocytes, cytokine production and increased expression of PD-1, an inhibitory molecule of T cells (72). Furthermore, studies have shown viral re-activation of CMV and HSV in critically ill patients who were previously healthy from an immune perspective (73). Unfortunately, over 12 RCTs comparing G-CSF to GM-CSF have not shown any evidence to support the use of either growth factor to help overcome the immunosuppressive state of septic patients (74). The effects of a sepsis induced immune

paralysis continue to be investigated in hopes to overcome the associated morbidity and mortality (46).

The reality is that a dual phase septic model represented by SIRS and CARS is an oversimplification of the pro- and anti-inflammatory cytokine balance. Both pro- and anti-inflammatory cytokines are released concurrently in the early phase of sepsis (75). In the early phase the pro-inflammatory cytokine cascade predominates, however anti-inflammatory cytokines such as IL-10 are already elevated (76). The most up to date understanding is that in early and late sepsis there are potentially extreme phenotypes of SIRS and CARS, however within the spectrum of sepsis there is a mixed anti-inflammatory response syndrome (MARS) (27, 77). Importantly, genome-wide transcription profiling of IL-10 and TNF-a in 784 patients failed to provide any evidence of a dual phase sepsis model (78). As sepsis progresses, pro- and anti-inflammatory cytokine concentrations dynamically change. Recently, no predominate pro- or anti-inflammatory cytokine phase has been found to contribute to sepsis mortality (79). This complexity of cytokine interaction highlights the incompletely understood pathophysiology of sepsis (80).

The classic pro-inflammatory cytokines are TNF-a, IL-1, IL-6, IL-8, IL-12 and INF-y (81). TNF-a and IL-1 are endogenous pyrogenic cytokines that are central to triggering symptoms of elevated temperature and tachycardia (82). TNF-a gene expression is increased via LPS/TLR4 induced activation of NF-kB. TNF-a is up regulated within minutes, has a short half-life and levels are undetectable within 3-4 hours (83). TNF-a induces a positive feedback loop by activating other pro-inflammatory mediators such as but not limited to: platelet-activating factor (PAF), IL-1, IL-2, IL-6 and

IL-8. TNF-a is released by neutrophils, endothelial cells and macrophages, which elicit further release and production of pro-inflammatory cytokines, adhesion molecules and chemokines (84). Through soluble TNF receptors, TNF-a also activates the immune system via programmed cell death (apoptosis) in order to remove invading pathogens that have undergone phagocytosis. The presence of elevated TNF-a activates anti-inflammatory cytokines IL-10 and HGF as counter-regulatory mechanisms to prevent an over-exaggerated pro-inflammatory response from occurring (85).

IL-6 is a pleiotropic cytokine activated by TNF-a and is responsible for increased production of acute phase proteins, inflammation, mucosal barrier dysfunction and endothelial injury (86). Interestingly, it has anti-inflammatory properties enhancing levels of IL-R1a, IL-10 and soluble TNF receptors (84). Clinical and animal studies have demonstrated that increasing levels of IL-6 correlate with disease severity and mortality (87, 88). However, overall clinical applicability of IL-6 as a biomarker remains undetermined (89). Macrophages, endothelial cells, and epithelial cells secrete IL-8. Its major functions are to induce IFN-y secretion and act as a chemokine targeting neutrophils to sites of inflammation and infection (90). IL-17 is a recently discovered pro-inflammatory cytokine that is involved in maintaining intestinal integrity, gut homeostasis and can indirectly increase IL-8 levels (91).

RANTES, MCP-1, MIP-1a and MIP-1b are all part of a major group of chemokines that share a similar structure and are able to recruit monocytes, macrophages and T cells (92). These chemokines are actively involved in sepsis. In LPS induced sepsis peritoneal macrophage mRNA expression is increased for RANTES, MCP-1, MIP-1a, MIP-1b and chemokine expression is specifically increased in the liver (93). Sepsis

induced acute lung injury is associated with increased concentrations of RANTES and MIP-1a, which are dependent on nuclear transcription and activation of Nf-KB (94).

RANTES is produced by macrophages, epithelial cells, platelets, eosinophils, T lymphocytes and it is also chemotactic for neutrophils, macrophages, eosinophils, basophils, dendritic and NK cells (95). Its role in animal models of sepsis has been established, however the clinical significance of RANTES in adult clinical studies is yet to be elucidated. In a CLP model of abdominal sepsis RANTES increases levels of IFN-y and correlates with mortality (92). Platelets are recruited to sites of inflammation and are a primary source of RANTES (96) and the release of RANTES via platelets is a major regulator of pulmonary neutrophil recruitment and lung injury (97).

The anti-inflammatory and immune regulating cytokines are IL-1Ra, IL-2R, IL-4, IL-7, IL-10 and IL-13. IL-1Ra is the inhibitory receptor of IL-1. IL-2R is required for T cell maturation and regulation (98). IL-2R shares a common receptor chain with IL-4, IL-7 and other cytokines that control T cell function (99). Lymphopenia triggers IL-7 release, which is responsible for T cell homeostasis, and proliferation (100). IL-7 is also able to overcome effects of lymphocyte apoptosis (101). IL-10 is a pleiotropic cytokine produced by almost all leukocytes and is the most critical counter regulatory cytokine controlling anti-inflammatory responses and immunosuppression (102). IL-10 acts through several receptors controlling various functions, however the direct actions of IL-10 are geared towards APCs (103). In sepsis, IL-10 functions by inhibiting LPS induced pro-inflammatory cytokines via STAT3 dependent and independent mechanisms. STAT3 is a signal transduction protein and is activated by IL-10.

IL-10 dependent STAT3 activation induces genes that suppress pro-inflammatory cytokines such as TNF-a (104). STAT3 and LPS/TLR4 induced NF-kB bind to IL-1Ra and increase IL-1ra transcription, promoting an anti-inflammatory environment (105). In animal models, the process of STAT3 activation and eventual suppression of pro-inflammatory cytokine expression requires approximately 4 hours (105). STAT3 deficient macrophages are more sensitive to LPS induced sepsis and produce elevated levels of TNF-a, IL-1b, IL-6, IL-12 and IFN-y, highlighting the critical role of STAT3 controlling the pro-inflammatory cascade (106).

A relatively new finding of IL-10 is that inhibition of TNF-a translation is mediated by STAT3 independent mechanisms with a quick onset of action and does not require transcription (107). Interestingly, IL-10 is able to restore endothelial dysfunction by inhibiting TNF-a induced activation of NF-kB (108). TNF-a is also inhibited by IL-10 via different signaling pathways in early and late phases of sepsis (109).

Overall, IL-10 inhibits leukocyte production of; pro-inflammatory cytokines, chemokines (IL-8 MCP-1, MIP-1a, MIP-1b and RANTES), and; growth factors (GM-CSF, G-CSF); furthermore, it enhances IL-1Ra production and inhibits antigen presentation of APCs (102, 110). In animal models of sepsis elevated levels of IL-10 are correlated with improved survival (111, 112) and control the development of septic shock (113). Absent levels of IL-10 are responsible for the development of autoimmune diseases and spontaneous colitis, highlighting the importance of IL-10 on inflammatory homeostasis (114).

1.2 Abdominal sepsis and surgery

Abdominal sepsis may be the result of several etiologies including bowel or colonic perforation, ischemia, anastomotic leaks and others. Peritonitis is used interchangeably with abdominal sepsis and refers to inflammation of the peritoneal cavity, usually from infection. Local immune cells in the abdomen induce a pro-inflammatory cytokine response.

Peritonitis is classified as primary, secondary or tertiary (115). Primary peritonitis is the result of a spontaneous infection that develops within the sterile peritoneal cavity. It is usually seen in patients on peritoneal dialysis. Secondary peritonitis is due to organ injury or infection within the abdomen that allows bacteria to enter into a sterile peritoneal cavity. Secondary peritonitis is the principle indication for surgery. Tertiary peritonitis usually occurs following primary or secondary peritonitis in critically ill patients. It is represented by recurrent infections that were previously treated, or new difficult to treat and eradicate infections.

The peritoneal cavity is a sterile environment covered by a single celled lining composed of mesoderm and epithelium termed mesothelium (116). In response to infection, the peritoneum activates the diaphragm as a pump, initiates innate and specific immune systems and forms abscess pockets to keep infection localized (117). The omentum acts as a blanket protecting the intra-abdominal organs and intestinal tract. It contains metabolically active mesothelial cells (118), secondary lymphoid organs known as milky spots (119), and high endothelial venules (HEVs), (120) which interact to fight infection. In non-stressed environments milky spots contain (70%) macrophages, (10%) B lympthocytes, (10%) T lymphocytes and some mast cells (117). Milky spots have

dense capillary networks and provide a direct pathway to areas of inflammation in response to chemotactic cytokines. The gut is a known source and initiator of sepsis (121). During gastrointestinal tract perforation, the omentum naturally attempts to contain the perforation to prevent any further contamination. The omentum depends on mesothelial cells and peritoneal macrophages for the initiation of pro-inflammatory responses, immune cell recruitment and activation of T cells.

Mesothelial cells are considered non-professional antigen presenting cells (122). They express several different TLRs, release pro-inflammatory cytokines, chemokines and growth factors. Mesothelial cells are activated by IFN-y or TFN-a and they secrete: TNF-a, IL-6, IL-8, IL-15, MCP-1, MIP and RANTES (117, 123, 124). In the presence of TNF-a and IFN-y, CD40 is up regulated on mesothelial cells inducing increased expression of IL-15, RANTES and MCP-1 (125). Early in the response to infection mesothelial cells release IL-8, which is highly chemotactic and helps recruit neutrophils to the peritoneum. RANTES, MCP-1 and IL-15 attract and activate monocytes and T cells. Once mesothelial cells increase secretion of RANTES and MCP-1, IL-8 activity is decreased, resulting in reduced recruitment of neutrophils. Neutrophils travel along high endothelial venules (HEVs) located within the omentum and act as a direct pathway for neutrophils travelling to sites of inflammation (120). Leukocytes are recruited from blood vessels to the sites of infection via intra-cellular adhesion molecule-1 (ICAM-1) and other similar adhesion molecules (126).

In addition to activated immune cells producing cytokines in the local environment, the peritoneal fluid itself activates neutrophils (127). The peritoneum attempts to remove the highly inflammatory peritoneal milieu into the systemic

circulation. Peritoneal fluid is removed from the abdominal cavity to the systemic circulation via lymphatic channels called stomata that underlie the diaphragm (122). Stomata actively increase in size during inflammation. Once in the systemic circulation the peritoneal inflammatory milieu is able to travel through the respiratory, cardiac and renal systems and induce SIRS. With systemic re-distribution of such pathogenic and inflammatory milieu from the peritoneal cavity it is not surprising that severe abdominal sepsis/septic shock may lead to multi-organ failure.

It has been suggested that removal of peritoneal fluid and thereby peritoneal inflammatory cytokines reduces the development of multi-organ failure and ultimately improves survival (127). Kubiak et al. [128] confirmed this suggestion in porcine models of peritonitis managed with negative pressure wound therapy (NPWT). Similar animal models have shown reduced severity of acute lung injury (129) that is attributed to the removal of ascitic fluid containing inflammatory cytokines (130). Human observational studies with secondary peritonitis have found very concentrated peritoneal cytokine concentrations that are several times higher than the respective serum concentrations (131).

The peritoneal cavity is very active in its role off infection, and molecularly responds similarly as the systemic response does to pathogens. Abdominal surgery itself causes tissue damage, which results in the release of DAMPs and activation of the innate immune system. Release of endogenous DAMPs activates similar signaling cascades in sepsis and can result in post-operative SIRS. TNF-a, IL-6 and IL-8 are increased immediately post-operatively in elective abdominal surgeries (132). Measurement of peritoneal and serum cytokines have shown promise in measuring the development of

post-operative complications after elective procedures. Normal post-operative course following abdominal surgery is reflected with decreasing peritoneal TNF-a, IL-6 and IL-10 concentrations over time (133). Increased post-operative peritoneal TNF-a concentrations following abdominal surgery have been shown to correlate with post-operative complications (134). Animal models of peritonitis with elevated peritoneal cytokines are associated with increasing mortality (135). However, in patients with secondary peritonitis managed with single staged laparotomy no significant differences in concentrations of peritoneal TNF-a, IL-6, IFN-y and IL-10 between survivors and non-survivors have been found (136).

Abdominal sepsis prior to the advent of source control and laparotomy was managed conservatively and was a fatal disease. Large volumes of peritoneal contamination cannot be locally controlled by the omentum. With the introduction of surgical intervention mortality rates have dramatically decreased although emergency laparotomy continues to have high morbidity and mortality (137). Furthermore, delay to intervention of 24hrs or more results in significantly increased patient mortality (138, 139). The traditional approach in emergency surgery for trauma and abdominal sepsis emphasizes source control with definitive repair during a single staged operation (140). The abdominal fascia is re-approximated primarily with sutures, this is known as primary abdominal closure (PAC). For any clinical de-compensation secondary to abdominal sepsis post-operatively, patients are brought back to the operating room for re-laparotomy and source control. This management pathway is known as on-demand laparotomy. Single staged laparotomy is considered the gold standard of surgical management, however it is inadequate for severe abdominal sepsis (141).

Significant hemorrhage requiring abdominal packing, gross peritoneal contamination, hemodynamic instability and abdominal compartment syndrome (ACS) are examples in which traditional single staged procedures cannot successfully be utilized (142). Trauma patients managed with single staged procedure with a combination of acidosis, coagulopathy and hypothermia have poor outcomes and would benefit from damage control laparotomy (143, 144). Prolonged definitive operations with deranged physiology are associated with severe complications and increased mortality.

ACS is the result of increased abdominal pressure causing secondary low tidal volumes, decreased venous return, worsening renal function and increased ventilation pressures, which ultimately lead to multi-organ failure (145). This constellation of signs and symptoms may occur prior to or after definitive surgery. ACS may develop primarily or secondarily (146). Primary ACS is the result of organ dysfunction or trauma in the abdominal/pelvis regions. Secondary ACS results from indirect abdominal/pelvic damage such as, massive fluid resuscitation prior to or after a surgical procedure.

Traumatic and septic pathologies that lead to significant patient instability and deranged physiology highlight the need for abbreviated laparotomy, open abdomen and ICU resuscitation prior to definitive management (147). During the 1990s, in the field of trauma surgery this management pathway developed into a formalized concept known as, " damage control surgery" (DCS) (148). The goals of DCS for abdominal sepsis are medical stabilization, abbreviated laparotomy with source control, ongoing resuscitation within the ICU, prevention of ACS and planned re-laparotomy for re-evaluation of the abdominal cavity (149-151). The abdominal fascia is not re-approximated (open abdomen) and a temporary abdominal closure (TAC) is utilized to protect abdominal

contents until a subsequent planned-re-laparotomy can be performed approximately within 48-72 hours.

The concept of DCS for severe abdominal sepsis originated in the 1980s under the term "Etappenlavage" at approximately the same time as the DCS concept was being formalized in trauma (152). Etappenlavage described the process of planned re-laparotomies at regular intervals to ensure macroscopic clearance of disease (153). Interestingly, open abdomen management for abdominal sepsis was first described in 1897 and 1940 (154). In 1994, Wittmann explored the role of SCL with a temporary bridging fascial closure technique followed by planned re-assessment of the peritoneal cavity 48-72 hours later. On re-assessment, decisions were made regarding the need for additional lavage, debridement and or/definitive closure. Wittmann identified that open abdomen with a staged approach had a significantly lower mortality rate when compared to single staged operation (28.1% versus 44.2%) with both groups matched with the APACHE-II score (155).

Prior (and concurrently) to the adoption of open abdomen and the use of a TAC, some surgeons would perform planned re-laparotomies for severe peritonitis with closure of the abdomen at each laparotomy. In 1983, Penninck et al.[156] retrospectively found significantly lower mortality rates for planned re-laparotomy compared to single staged procedure for severe abdominal sepsis. Despite the early documented success of planned re-laparotomies (without open abdomen) the evidence was poor. In the 2002, a meta-analysis of 8 studies found no significant survival advantage between planned re-laparotomy and on-demand laparotomy (157).

In 2007 the first randomized trial compared planned versus on-demand laparotomy for secondary peritonitis and found no difference in mortality between the two approaches (158). The patients in the planned re-laparotomy group underwent primary abdominal closure at the initial source control operation with re-opening of the abdomen at planned intervals. Patients that required "imperative re-laparotomy" (i.e. gauze packing, stapled ends without re-anastomosis) were excluded.

During the early period of planned re-laparotomy the abdomen was closed at each interval laparotomy until the abdomen could no longer be closed, at which point the abdomen was packed with soaked gauze or non-absorbable mesh to prevent evisceration of abdominal contents. This marked the beginning of temporary abdominal closure (TAC) and the development of complications associated with early TAC devices in abdominal sepsis (159).

These cumulative results reduced early enthusiasm for planned re-laparotomy (with interval abdominal closure at each laparotomy) for the septic abdomen, and on-demand laparotomy was deemed to be the management strategy of choice. Enthusiasm for open abdomen and planned re-laparotomy was further damped when a second randomized control trial found no significant difference between planned re-laparotomy and on-demand laparotomy (160). In this study, the planned re-laparotomy group underwent open abdominal management with a sandwich technique, which was already known to have associated complications (161). In attempts to overcome complications of initial open abdomen management several TAC devices were utilized with varying degrees of success. This led to the development of an optimal TAC guide. The main

goals were to provide a rapid, safe entry into the abdomen, preserve abdominal fascia, provide undue tension and provide a homeostatic environment (162).

The evolution of the open abdomen and NPWT has also significantly altered the management of surgical abdominal sepsis (163-166). The use of NPWT as a TAC has become most commonly used because it has several optimal TAC qualities. Perhaps most importantly, it removes peritoneal effluent. The V.A.C. was a commercially available NPWT that displayed good results with case series in severe peritonitis (167, 168). The NPWT techniques of ABThera and Barker method were recently compared in a RCT for abdominal sepsis and trauma patients requiring open abdomen (169). Interestingly, there was no significant difference between ABThera or barker method groups for peritoneal or serum cytokines that were drawn during planned re-laparotomy at 24hr or 48hrs. The study design was not optimized for the evaluation of cytokine concentrations in abdominal sepsis because there were an equal amount of trauma and sepsis patients in each group. Although trauma elicits a SIRS response and shares similar signaling pathways, there is no evidence to suggest that the magnitude of the inflammatory response is equal.

Holzheimer et al. [170] in 1995 compared peritoneal and serum pro-inflammatory cytokines in severe abdominal sepsis patients who underwent planned re-laparotomy. They found that peritoneal concentrations of TNF-a and IL-6 were several times higher than their systemic counterparts. Interestingly, survivors had higher peritoneal concentrations of IL-6 compared to non-survivors. Frohlich et al. [171] identified that in secondary peritonitis peritoneal irrigation with 10L of Ringer's solution was able to

reduce peritoneal concentrations of TNF-a and IL-8, however over time cytokine levels increased.

There is limited knowledge regarding the role and pattern of inflammatory cytokines in patients with severe abdominal sepsis/septic shock requiring laparotomy and open abdomen management. The inflammatory cytokine patterns and interactions need to be further explored in a practical clinical model. Translation of bench side knowledge to clinical practice is difficult due to; the inability to control clinical settings as in animal models, heterogeneity and size of human sepsis cohorts. Human studies evaluating relationships and prognostics of inflammatory cytokines during abdominal sepsis requiring laparotomy have been variable. Further research is required to determine the impact of negative pressure dressings on the pathophysiology of severe abdominal sepsis/septic shock requiring laparotomy.

1.3 Cytokine Biomarkers

Cytokines have been investigated extensively for their potential role as biomarkers in sepsis (89, 172-174). Despite many animal and human studies and over 170 biomarkers evaluated (175, 176) there is no consensus cytokine that is clinically available to help guide sepsis diagnosis, stratification, or management of sepsis (177). Pro-calcitonin is perhaps the best well-known inflammatory marker to separate septic from non-septic individuals, however its clinical utility is variable, and its not utilized widely (178). The interest in cytokines as biomarkers arose when research began identifying the SIRS response in sepsis and the associated pro-inflammatory cytokine storm. Not only was there an interest in biomarkers, there was enthusiasm to find inhibitors of pro-inflammatory cytokines and receptors (179). It was believed that

inhibition of major pro-inflammatory cytokines such as TNF-a would help reduce mortality in sepsis. As mentioned previously, over twenty studies have failed to identify significant reductions in mortality with pro-inflammatory cytokine or TLR4 inhibitors. Interestingly, TNF-a and IL-1 inhibitors are used in inflammatory bowel disease as a mainstay of treatment (83). The failure of identifying a singular inhibitor of the pro-inflammatory response demonstrates the complexity of pathways that result in transcription of pro-inflammatory mediators.

Biomarker studies have identified that simultaneous evaluation of several cytokines may show promise (180-182). However, many of these studies are heterogeneous (183-185), assess different cytokines (186, 187), and have variable results at different time points during hospital admission (188, 189). It thought that the anatomic location of sepsis elicits different inflammatory cytokine profiles (190). Furthermore, no single biomarker has been shown to predict sepsis in polytrauma patients (191). Unfortunately, there are no cytokine profiles for different anatomic locations. Promising biomarkers do exist, however large studies with consistent methodology, cutoff ranges and homogeneous populations are required (192).

1.4 Objectives

The primary objectives were to determine if peritoneal or serum cytokines could support or guide the use of VAC and differentiate severity of disease in patients with severe abdominal sepsis/septic shock requiring urgent source control laparotomy. The secondary objectives were to identify if serum or peritoneal cytokines could predict mortality, and evaluate longitudinal cytokine trends in planned re-laparotomy.

CHAPTER 2: Methods

2.1 Prospective study design

This was an observational prospective case series performed at VGH from December 2011 to June 2013, and was approved by the UBC ethics review board.

Patients were included if there was evidence of severe sepsis or septic shock with a suspected or known abdominal source of infection, and if urgent source control laparotomy was required. Intra-operatively, during the initial SCL the staff surgeon determined the decision for PAC or VAC based on the degree of abdominal contamination, clinical status of the patient, and surgeon experience. There was no designed study protocol to determine closure status at the time of initial SCL. There was no formal documentation or questionnaire provided to the attending surgeon to identify specific reasons for selecting either PAC or VAC management pathways. No placebo was provided, no medications were withheld and no alternative treatment was provided. Potential patients were identified and screened for inclusion criteria. If inclusion criteria were met and consent was obtained patients were included into the study.

Inclusion criteria

Definitions of severe sepsis and septic shock were defined as per International sepsis consensus definitions (8, 193). Severe sepsis was defined as at least one finding of SIRS criteria (WBC<4 or >12 x 10^3/*u*L, Temperature < 36 or > 38.2 degrees Celsius, heart rate > 90 beats per min, respiratory rate > 20/min) along with evidence of altered mental status or organ dysfunction defined as; arterial hypoxemia (Pa02/Fi02 < 300), urine output < 0.5 ml/kg/hr, creatinine increase > 0.5mg/dL, ileus, platelets < 100,000, hypo-perfusion (lactate > 1mmol/L, clinical "mottling") or hypotension (systolic BP < 90

mmHg) responsive to fluid resuscitation. Septic shock was defined as hypotension that was non-responsive to a fluid challenge of 30ml/kg bolus (or 1L fluid challenge), MAP < 60 mmHg, or use of vasopressors.

Exclusion criteria

Patients less than 18 years of age, sepsis secondary to trauma, laparoscopy without conversion to laparotomy, laparotomy for non-septic indications, and abdominal sepsis without source control laparotomy were excluded. Cases were excluded if the attending surgeon intra-operatively at the time of primary source control laparotomy deemed that the degree of the abdominal insult was non-survivable.

2.2 Measure of disease severity

Severity of disease was measured using the APACHE-IV score and predicted mortality rate (PMR). The APACHE-IV is a tool used to stratify disease severity by PMR and predict ICU length of stay (LOS) (194). The APACHE-IV produces a score and PMR based on an integration of data including but not limited to diagnosis, clinical/laboratory data and age. The "worst" values within the first 24 hours of ICU admission are inputted into the calculator and a PMR is produced. This is a one-time calculation. We used the Cerner APACHE-IV calculator (195) (which is readily available online) and calculated the PMR for each patient according to the Cerner protocol based on prospectively collected data. Despite two patients not admitted to the ICU for abdominal sepsis we calculated the APACHE-IV to have a baseline of severity of disease for cohort groups.

Patients who met inclusion criteria and required urgent source control laparotomy were termed surgical abdominal sepsis (SABS) patients and were further categorized into

two distinct groups based on abdominal closure method at initial source control laparotomy.

2.3 Surgical management definitions

- Primary abdominal closure (PAC) - Re-approximation of abdominal fascia using sutures at the primary source control surgery. The decision was made by the attending surgeon and was dependent on physiologic status of the patient and the ability to perform definitive source control (debridement or resection of infected/necrotic tissue, lavage, bowel resection and anastomosis, etc.)
- Vacuum assisted closure (VAC) – following initial source control procedure, decision was made intra-operatively at the discretion of the attending surgeon to proceed with an open abdomen (abdominal fascia not re-approximated) and temporary abdominal closure device. The temporary abdominal closure was managed with a negative pressure dressing and the vacuum assisted closure device (VAC) was used if available. Common indications for VAC were: hemodynamic instability, ongoing volume resuscitation, gross peritoneal contamination, anticipated abdominal compartment syndrome, bowel edema, and/or loss of abdominal domain. Patients with VAC returned to the operating room for re-assessment of the peritoneal cavity within 48-72 hours of initial source control laparotomy unless patient instability required earlier operative intervention. The process of re-assessment in the operating room was repeated until the abdomen was macroscopically clear of sepsis, source control definitively achieved and assessed by attending surgeon. At this point the abdominal cavity was suitable for definitive closure.

- Planned re-look laparotomy: re-assessment of the peritoneal cavity after initial source control laparotomy and VAC application.

2.4 Sample collection and processing

Blood samples were obtained pre- and post-operatively via an arterial line in the operating room prior to the start of initial source control laparotomy. If arterial line was not available, venous samples were used. Pre-operatively, blood samples were obtained within an hour of abdominal incision and post-operatively samples were taken within an hour of procedure completion. Peritoneal fluid samples were obtained with a 10 cc syringe immediately after laparotomy incision was made and prior to any significant intra-abdominal manipulation or definitive source control. All samples were kept on ice and centrifuged within one hour of collection.

Pre- and post-operative blood, and peritoneal fluid samples were spun at 1500 G for 5 minutes; supernatant was collected and stored at -70 degrees Celsius. Serum and peritoneal fluid samples were analyzed with a Luminex Human Cytokine 30-PlexPanel from Life Technologies. Observed cytokine concentrations were reported at pg/ml. If a specific sample's observed cytokine concentration was out of the detection range, the maximum or minimum observed cytokine concentration value was reported. In a few samples IL-6 concentrations in serum and peritoneal fluid were higher than the upper limit of detection.

The majority of patient's next of kin/decision makers signed informed consent for blood and peritoneal samples over the patient themselves. This was due to the clinical status of the patient and due to the limited time available to identify and consent patients undergoing an urgent/emergent operation.

2.5 Statistical methods

Analysis was stratified into VAC and PAC groups. Descriptive statistics were used to summarize VAC and PAC groups. Cytokine concentrations for all groups expressed with median values. Serum and peritoneal fluid samples were compared between PAC/VAC and non-survivor/survivor groups with the mean ranks Mann-Whitney U test. Wilcoxon signed ranks test was used to compare pre- and post operative serum cytokines for VAC, PAC, survivors and non-survivors independently. Open abdomen with and without necrotizing pancreatitis was compared to the PAC group with the Kruskal-Wallis test. Significance was defined as alpha of less than 0.05. The 2-tailed test for p values was used. All statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) Version 20.0 for Macintosh computers. (196).

CHAPTER 3 Results

We identified 16 patients however 4 families refused to consent their family member to participate in the study. Twelve patients (4 PAC and 8 VAC cases) were included. Patient demographics and sepsis etiologies are listed in **table 1.** Observed PAC mortality was 25% and 3 of 4 PAC patients were diagnosed with septic shock prior to SCL. Observed VAC mortality was 37.5% and all VAC cases were diagnosed with septic shock prior to primary SCL. APACHE-IV PMR was 47.6% and 43.3% for PAC and VAC. 10 patients were admitted or returned to the ICU secondary to abdominal sepsis. One gastro-intestinal perforation was managed with PAC, kept in the post-operative anesthesia care unit requiring pressors for a short period of time and was not admitted to the ICU. A rectal perforation was managed with PAC and was not admitted to the ICU post-operatively. Within 24 hours there was a post-operative complication of hemorrhage, which required an on-demand laparotomy with VAC management. Following on demand laparotomy for hemorrhage the patient was admitted to the ICU. APACHE-IV PMRs were calculated and included for all patients in order to compare severity of disease between the PAC and VAC groups. Within the entire cohort, there were 4 cases with presence of an underlying malignancy.

We found no significant difference between VAC and PAC when comparing both pre-operative and post-operative serum cytokine concentrations (**Table 2,3)**. In order to identify potential cytokine trends during the pre-and operative course for each VAC and PAC group we calculated a mean delta score (post-operative minus pre-operative serum concentrations). **figures 1 and 2.**

Comparing serum prior to and post primary source control laparotomy in the VAC cohort, significant post-operative elevations were found for RANTES ($p = 0.025$) and IFN-y ($p = 0.028$) **Table 5.** IL-10, MIP-1b, and EGF all trended towards a significant increase post-operatively with p values equal to 0.05. No significant differences were found between pre- and post-operative serum cytokine concentrations at primary source control laparotomy for the PAC group.

Peritoneal cytokine concentrations of IL-6, IL-17, IL-5, and HGF in the VAC group were significantly higher compared to PAC at primary SCL $p<0.05$ (**Table 4)**. In the VAC group peritoneal cytokine concentrations at second SCL (7 cases) were compared to primary SCL (8 cases) and significant differences in IL-10, HGF and MIP-1a were identified ($p <0.05$) **Table 6**. Peritoneal cytokine concentrations in VAC patients at the primary SCL were compared to the third SCL (second re-laparotomy) of which there 5 cases. TNF-a and MIP-1b were elevated at the third laparotomy compared to the initial damage control laparotomy ($p<0.05$), while peritoneal IL-2R was higher at the first SCL compared to the third SCL ($p<0.05$). **Table 7.**

To assess the inflammatory process of necrotizing pancreatitis we specifically separated the necrotizing pancreatitis cases from other VAC sepsis etiologies and compared peritoneal cytokine concentrations to three groups (VAC with and without necrotizing pancreatitis and PAC) **Table 8.** The VAC group without necrotizing pancreatitis had significantly increased peritoneal cytokine concentrations of IL-2, IL-17 and IFN-y at primary SCL ($p<0.05$)

Pre-operative serum RANTES at primary SCL was significantly higher in all survivors compared to all non-survivors ($p = 0.042$) **Table 9**. No other significant

cytokine differences between survivors and non-survivors were identified in the serum of pre-operative primary SCL**.** In serum post primary SCL no significant cytokine concentration differences between all survivors and non-survivors were identified **Table 10**. No significant differences of peritoneal cytokine concentrations at primary SCL were identified between all non-survivors and survivors **Table 11.**

In subgroup analysis of all non-survivors there was 1 PAC and 3 VAC cases. No significant pre and post-operative serum or peritoneal cytokine concentration differences were identified. In subgroup analysis of all survivors there were 3 PAC and 5 VAC cases. Peritoneal cytokine concentrations of IL-6, IL-5, HGF, VEGF and FGF-b all trended to be almost significantly elevated in VAC survivors compared to PAC survivors. Post-operative serum of IL-13 also approached statistical significance **Table 12.**

Peritoneal concentrations at the second SCL were compared between all VAC survivors and non-survivors. Pre-operative serum concentrations of VEGF, FGF-b, IL-1b, IL-5, IL-4, IL-7 (**Table 13**) and post-operative serum concentrations of VEGF and IL-7 (**Table 14**) were all significantly elevated in VAC non-survivors.

CHAPTER 4 Discussions

During the course of our prospective case series we included 12 patients (4 PAC and 8 VAC cases) for study analysis. Our patient cohort developed severe abdominal sepsis/septic shock and required source control laparotomy. Serum and peritoneal samples taken at primary SCL represent, "Tx", an unknown point in time of the inflammatory cytokine response in relation to the inciting septic event. Clinically, Tx represents a time point in which septic patients despite medical treatment deteriorated and required urgent source control surgery. We assume that Tx represents a peak pro-inflammatory cytokine response and that we are capturing patients in the early phase of surgical abdominal sepsis. We do not have a measured cytokine level at the "T0" starting point of sepsis. Therefore we cannot calculate a delta cytokine concentration from Tx to T0 and establish a rate of cytokine change over a period of time. A T0 would have potentially improved our understanding of changes that influence clinical symptoms and disease progression from medical to surgical phases of sepsis.

The decision to measure both pre- and post-operative cytokine concentrations at each source control laparotomy was made for several reasons. We assumed that just prior to undergoing SCL patients would be at or close to the "peak" pro-inflammatory response. Just prior to surgery patients had decompensated clinically despite maximal medical treatment to the point that urgent/emergent surgery was needed. Measuring cytokines just prior to source control surgery would represent a patient's inflammatory status secondary to sepsis and would not be convoluted by the inflammatory impact of surgery. Measuring serum cytokines post-operatively provided the ability to measure the effect of surgery on the systemic inflammatory response by subtracting values from pre-

operative serum concentrations. Surgery is known to alter inflammatory cytokines and calculating the effect of source control surgery on both PAC and VAC groups would potentially provide valuable information. Measuring peritoneal fluid concentrations at primary SCL provided the ability to profile the local inflammatory response. It could also potentially help differentiate between severity of abdominal sepsis and support surgical decision-making.

There is variable consistency regarding information known about inflammatory cytokine relationships within the peritoneum. We casted a wide net by analyzing 30 cytokines, chemokines and growth factors in order to identify possible cytokine relationships in patients with severe abdominal sepsis requiring source control laparotomy. Despite a small sample size we were able to draw significant conclusions from the data. Our study confirms previous findings in animal sepsis models while providing new insight into pro- and anti-inflammatory cytokine relationships at primary SCL, second and third SCLs.

We hypothesized that patients with a more clinically severe abdominal sepsis would be represented by VAC and would have increased peritoneal and pre-operative serum cytokine concentrations in comparison to PAC. We assumed serum cytokines would be elevated secondarily to a strong inflammatory peritoneal milieu. We further hypothesized that patients with a clinically worse severe abdominal sepsis would have higher elevations of cytokines in peritoneal fluid. In an attempt to reduce the concentration of peritoneal cytokines, and intra-abdominal edema (prior to the development of ACS) the diaphragm would actively pump and transfer peritoneal fluid systemically via lymphatic drainage and secondarily induce a SIRS response pre-

operatively. We assumed that in the PAC group, intra-abdominal inflammatory fluid would not induce such a strong SIRS response because the PAC group would have a lower degree of severe abdominal sepsis, therefore lower peritoneal cytokine concentrations. Furthermore, we hypothesized that strongly elevated peritoneal concentrations at primary SCL would provide a biochemical indication for VAC management in order to reduce the local inflammatory milieu by removing excessive extravascular peritoneal fluid, and therefore reduce negative impact of elevated systemic cytokines.

In the VAC group we expected that peritoneal pro-inflammatory cytokine concentrations would be reduced yet still detectable at the first re-look laparotomy compared to primary SCL. We also hypothesized that cytokine concentrations (pro- and anti-inflammatory) would be reduced or unchanged in post-operative serum compared to pre-operative serum for the PAC group at primary SCL. Patients undergoing single staged laparotomy theoretically should not clinically decompensate intra-operatively and be able to tolerate definitive repair. Therefore, there would be a minimal impact on the distribution of cytokines systemically. In regards to VAC, we hypothesized that post-operative cytokines would be unchanged or elevated secondary to the impact of damage control surgery and potential clinical deterioration intra-operatively. Based on observations from previous studies we anticipated that peritoneal IL-6 and IL-10 concentrations would significantly differentiate between PAC/VAC and non-survivors versus survivors.

The most notable finding of our study confirmed our primary hypothesis and identified new relationships of peritoneal cytokines in humans with severe abdominal

sepsis requiring SCL. Peritoneal fluid from the VAC group contained significant elevations of IL-6, IL-17 IL-5, and HGF compared to the PAC group at primary SCL. These findings provide biochemical evidence that at the time of primary SCL, patients who underwent VAC management had a more hostile peritoneal cavity and a worse degree of severe sepsis/septic shock. These findings also provide new insight into the complex interaction of the mixed anti-inflammatory response in surgical abdominal sepsis. To our knowledge, this is the first porcine or human abdominal sepsis study have documented the simultaneous elevations of IL-6, IL-17, IL-5 and HGF in peritoneal fluid.

The major initiating cytokine of the pro-inflammatory response is TNF-a, which induces release of IL-6 and other pro-inflammatory cytokines. IL-6 and other pro-inflammatory cytokines promote endothelial damage and capillary leak through different signaling mechanisms (197, 198), which contribute interstitial edema, multi-organ dysfunction and distributive shock. Based on the short half-life of TNF-a we did not expect to find elevated concentrations within the serum or peritoneal fluid. IL-6 has a longer half-life and as previously discussed has several functions. Therefore we expected IL-6 to significantly contribute to the inflammatory response within the peritoneal cavity.

IL-6, IL-23 and IL-12 are able to activate Th17 T cells, a subset of T cells located within the intestinal tract, which function to maintain mucosal integrity, protect against invading organisms and maintain gut homeostasis (199). Intestinal T cells of the adaptive immune system protect the gut from exaggerated inflammatory responses due to invading pathogens. T cells are located in lymphoid organs of the GI tract (Peyers patches, mucosal associated lymphoid tissue), lamina propria and mucosal epithelium (200). Once Th17 cells are activated they secrete cytokines IL-17, IL-21 and IL-22 (201).

Additionally, activated Th17 cells are able to secrete chemokines (IL-8, IP10, MCP-1), cytokines (TNF-a, IL-6, G-CSF, GM-CSF), acute phase proteins, tissue remodeling proteins, and anti-microbial proteins (91). IL-17 recruits neutrophils via IL-8 signaling and up regulation via G-CSF. In a mouse model of peritonitis IL-17 expression is increased in neutrophils via TLR9 in response to E. coli infection (202). IL-17 induces strong pro-inflammatory cytokine responses, causes excessive local tissue damage and breaks intestinal epithelium when over expressed. Th17 cells also are able to secrete IL-10 in attempts to control the local pro-inflammatory environment (203).

There is a double-edged sword effect with IL-17. Animal abdominal sepsis models have found increased concentrations of IL-17 within the peritoneum and blocking peritoneal IL-17 improves survival by decreasing neutrophil counts, TNF-a and IL-6 (204). Simultaneously blocking IL-17 weakens the intestinal barrier and increases susceptibility to bacterial infections (205). Some optimal level of IL-17 expression is required to fight infection, as evidenced by the stimulation of anti-bacterial proteins and acute phase proteins, which play a protective role in gut immunity. However, excessive production of IL-17 and neutrophil recruitment produces a hostile pro-inflammatory environment. Il-17 is only recently becoming a well-known driver of the inflammatory response in sepsis. It is mostly known for its role in autoimmune disease, where IL-17 antibodies are able to control symptoms in psoriasis and rheumatoid arthritis (206).

In addition to Th17 cells, eosinophils also play a role in maintaining mucosal integrity within the gut. IL-5 stimulated by LPS and GM-CSF activate eosinophils to release pro-inflammatory molecules and DNA protecting the gut from pathogenic invasion (207). Eosinophils are found in high numbers within intestinal lamina propria,

express several TLRs, up-regulate IL-6 and are responsible for maintaining intestinal mucosal integrity (208). Traditionally eosinophils were only associated with humeral immunity, parasitic infections and asthma, however knowledge about their role in immune regulation, anti-bacterial immunity and other functions is expanding (209).

Eosinophils contain several pro-inflammatory cytokines, chemokines and growth factors (210). Similar to IL-17, eosinophils are beneficial in helping to control infection, however at some undetermined concentration eosinophils exhibit deleterious effects by exacerbating the inflammatory response in association with other molecules. There is communication between Th17 cells, IL-5, eosinophils and neutrophils. IL-23 activates Th17 cells to produce GM-CSF, which in turn activates IL-5. This is known as the IL-23, GM-CSF axis, which activates eosinophils and is thought to play a role in chronic intestinal inflammation and damage to intestinal epithelium (211). However, it has been identified that in mice infected with citrobacter rodentium, leukocytes within the lamina propria increase levels of GM-CSF, which in turn elicit the release of pro-inflammatory cytokines and promote the clearance of bacterial infection (212). GM-CSF has a CD131 receptor that IL-5 is able to interact with, and promotes the release of mitochondrial DNA from eosinophils into the extracellular space (213). This signaling mechanism helps neutrophils identify, coat, bind and kill bacteria through NETs. As we can see, IL-5 in association with IL-17 and GM-CSF play important and complex roles in anti-bacterial defense.

The chemokines RANTES, MCP-1, MIP-1a and eotoxin-1 can also recruit and activate the degranulation of eosinophils causing the release of pro-inflammatory mediators that induce colitis in vitro studies (214). IL-33 is an alarmin, which functions

as a DAMP, can be found on endothelial cells and epithelial barrier cells (215). IL-33 is released secondary to necrotic cell death and stimulates innate lymphoid cells to produce large quantities of IL-5 and IL-13 (215). Even though these animal studies describe the role of IL-5 and eosinophils in non-septic inflammation, it is not surprising that we see significantly elevated IL-17 and IL-5 in the peritoneal fluid of VAC patients compared to PAC patients. Pro-inflammatory cytokines and PRRs activate eosinophils, neutrophils and macrophages. The over exaggerated activation of eosinophils can have a locally destructive effect on intestinal epithelial cells and contribute to the hostile peritoneal environment seen in abdominal sepsis.

Sepsis induces secondary tissue damage from pro-inflammatory cytokines, which can be countered by other cytokines aside from IL-10. HGF is a pleiotropic anti-inflammatory cytokine responsible for regeneration of damaged tissue (216), control of inflammation via down regulation of pro-inflammatory cytokines (TNF-a, IFN-y, IL-6), angiogenesis (217) and inhibition of apoptosis (218). HGF is most notable for its role in liver regeneration after hepatic insults such as liver resections and hepatic ischemia

HGF is found in an inactive state and in order to become activated it is cleaved by proteases of the coagulation cascade (219). Activated HGF is able to reduce the pro-inflammatory cytokine response initiated by TNF-a activated macrophages by interfering with NF-kB signaling, which also simultaneously induces HGF gene expression (220). In patients with SIRS, plasma neutrophils recruited to sites of inflammation release intracellular stored HGF (221). HGF released via degranulation from neutrophils is thought to help promote local wound repair (222). In our cohort, elevated HGF in peritoneal fluid of VAC patients is likely released secondary to the pro-inflammatory

environment in order to down-regulate pro-inflammatory mediators. In addition to the pro-inflammatory role of neutrophils, HGF is also released to promote wound healing and tissue regeneration (40).

As a potential biomarker, clinical studies have shown that HGF is elevated in patients with community-acquired infections (223, 224). Animal models of sepsis provide evidence that HGF can block the development of both acute respiratory distress syndrome and acute renal failure (225). These clinical and animal studies illustrate the pertinent role of HGF as an important anti- inflammatory cytokine in sepsis.

The significant elevation of peritoneal IL-6, IL-17, IL-5, and HGF in the VAC group provides novel evidence characterizing relationships between pro and anti-inflammatory cytokines in severe abdominal sepsis/septic shock requiring urgent SCL. Our findings indicate that HGF can be used as a biomarker for distinguishing severity of abdominal sepsis in this critically ill patient population.

VAC and PAC groups both had elevations of pro- and anti-inflammatory cytokines in the serum prior to primary SCL. Animal models of sepsis and human studies have shown that both pro- and anti-inflammatory cytokines are elevated in the early phase of sepsis (226). Our results provide human data that is consistent with these findings and support the model of a MARS in surgical abdominal sepsis. We found elevated pro-/anti-inflammatory cytokines and growth factors prior to the initial source control surgery. We did not have an initial T0 time frame, and therefore we cannot identify how truly elevated the serum cytokines were at primary source control surgery compared to T0. Perhaps as in previous findings, in the first few hours of sepsis only pro-

inflammatory cytokines can be detected in serum, as transcription of anti-inflammatory cytokines is slowly up regulated.

There was no significant difference in either pre-operative or post-operative serum cytokine concentrations at primary SCL between PAC and VAC group, which differed from our hypothesis. We calculated a mean delta by subtracting post-serum from pre-serum cytokine concentrations for each of the VAC and PAC cohorts. We did this in order to see if we could identify any trends that were not captured when comparing PAC/VAC serum cytokine concentrations at pre-operative and post-operative time points. The most obvious pattern identified was that VAC and PAC displayed opposite behaviors for certain pro- and anti- inflammatory cytokine concentrations. MIP-1a, IFN-y, IL-8, TNF-a, IL-12, MIP-1b, IL-10, IL-13, IL-1ra, and IL-5 all increased (non-significantly) in VAC and decreased in PAC group' serum post-operatively.

At the time of primary SCL the VAC group had significantly elevated post-operative serum concentrations of IFN-y and RANTES compared to pre-operative serum. MIP-1a, IL-10, and EGF trended to be higher in post-operative serum of the VAC group. While in the PAC group there was no significant increase in post-operative serum cytokines at primary SCL.

There are two possibilities to explain these results. The first possibility is that the VAC group had a SIRS response that had not yet reached peak inflammatory concentrations at the measured time point, and the local and systemic inflammatory response was increasing without an additional inflammatory effect of surgery. The second possibility was the effect of surgery that induced a pro-inflammatory response represented by increased serum levels of RANTES and IFN-y post-operatively. Another

explanation is that there was a combined effect of an increasing SIRS response from the combined septic insult and the inflammatory response secondary to surgery resulting in the significant elevations of RANTES and IFN-y.

It is also possible that the PAC group had a SIRS response that had already reached peak concentration just prior to SCL. In addition, the effect of surgery in the PAC group was minimal, accounting for no significant differences in serum pre or post-operatively at SCL. Lower peritoneal concentrations and reduced post-operative serum cytokine concentrations may indirectly indicate that patients who underwent PAC were clinically stable enough to undergo source control with a single staged procedure.

The second possibility discussed is based on the principles of sepsis compartmentalization (227) and damage control surgery. Compartmentalization of sepsis refers to the fact that cytokines and inflammation is produced at the local site of septic insult and that unaffected areas do not necessarily express the same levels or types of cytokines in response to the septic injury.

VAC cases may potentially have been at the peak SIRS response, remained locally within the peritoneum and did not produce significant systemic effects in the circulation. It's plausible that the local response contained the inflammatory milieu, elevated local concentrations of cytokines and prevented absorption of fluid from the peritoneum to the systemic circulation resulting in lower than expected pre-operative serum cytokines. However, it has previously been shown that gut derived inflammation induces SIRS and multi-organ dysfunction through mesenteric lymphatic drainage (228). Perhaps the peritoneal inflammatory milieu may not have had immediate access to the systemic circulation depending on the location of septic insult. Therefore, serum

cytokines were measured at an early time point prior to systemic elevation. Even though stomata increase in size during inflammation in order to remove inflammatory milieu out of the abdomen they are unevenly distributed throughout the peritoneal cavity (122), which potentially explains a delay in rising serum cytokines.

Lymphatic drainage of peritoneal fluid through the thoracic duct is an important communicator between the peritoneal cavity and systemic circulation. Animal models of sepsis have demonstrated that mesenteric lymph outflow from the abdomen is decreased with intra-abdominal hypertension (229) and mechanical ventilation with high PEEP (230). Ligating the thoracic duct impairs liver function and increases endotoxin in the portal veins (231). Human data in secondary peritonitis also suggests that peritoneal cytokine concentrations do not readily cross into the systemic circulation (171). In our study, perhaps mesenteric lymphatics were blocked secondarily to relative degrees of intra-abdominal hypertension. Unfortunately, intra-abdominal compartment pressures were not measured. These examples provide substantial reason as to why serum cytokines are not significantly elevated in VAC compared to PAC, despite significantly elevated peritoneal cytokine concentrations in VAC at primary SCL.

VAC cases underwent damage control surgery during primary SCL. The attending staff surgeons decided to employ open management of abdominal sepsis with a VAC dressing during the operation. As surgery was performed, the peritoneal contents were manipulated, and the localized infection was disturbed in attempts to achieve proper source control. This process could have also contributed to peritoneal inflammatory milieu re-distributing systemically and eliciting a strong inflammatory response post-operatively. As previously discussed, DCS is employed to obtain quick and efficient

source control and abbreviate the laparotomy as needed. This is due to the potential for clinical deterioration of patients on the operating room table. As the septic insult actively being fixed, patients often decompensate clinically as inflammatory milieu is opened and re-distributed systemically. Significantly increased local peritoneal and elevated post-operative serum cytokines in the VAC cohort support the concepts of sepsis compartmentalization, role of mesenteric lymphatic drainage and the effect of damage control surgery.

Peritoneal cytokine concentrations did not differentiate between survivors and non-survivors. It was surprising that IL-10 did not significantly differentiate between VAC versus PAC and non-survivors versus survivors due to its role in controlling the inflammatory response. Based on the significantly elevated peritoneal concentrations in VAC, we believe that VAC may have provided a survival advantage by removal of inflammatory peritoneal milieu throughout the duration of open abdomen management. Therefore, negative pressure wound therapy can be thought of as a disease-modifying agent. During the course of the open abdomen, negative pressure wound therapy potentially reduces elevated peritoneal cytokine concentrations re-distributing systemically and inducing or worsening multi-organ dysfunction. In a similar patient population retrospective data has shown a significant survival advantage with VAC management compared to PAC (232).

A potential reason why peritoneal cytokine concentrations did not differ between survivors and non-survivors is due to our small sample size. Future studies with increased numbers need to be performed in order to confirm our findings of elevated peritoneal

cytokines in VAC, the specific cytokine patterns identified and removal of inflammatory peritoneal milieu as a disease-modifying treatment.

We found that pre-operative serum concentration of RANTES was significantly elevated in survivors compared to non-survivors ($p < 0.05$). The chemokines MIP-1a, MIP-1b, and MCP-1 trended higher in pre-op serum in survivors compared to non-survivors ($p>0.05$). As previously mentioned, RANTES and IFN-y were significantly elevated in post-operative serum of VAC patients compared to pre-operative serum levels. This is an interesting finding. Elevated levels of RANTES have previously been found to correlate with mortality in neonatal sepsis (95). We also expected elevated RANTES concentrations within peritoneum or serum to be more closely correlated with non-survivors rather than survivors. This finding maybe an outlier, or RANTES as some protective effect in adult abdominal sepsis patients. A potential explanation is that survivors did not develop intra-abdominal hypertension as often as non-survivors and therefore systemic serum levels of RANTES and IFN-y were elevated. Alternatively, mesenteric lymph flow was non-obstructed allowing for inflammatory peritoneal milieu to be re-distributed systemically. It has previously been shown that surgical abdominal sepsis survivors have higher serum levels of IL-6 compared to non-survivors (233). These findings support the idea that up until a certain threshold, the body is able to tolerate and overcome elevated pro-inflammatory cytokines for survival in surgical abdominal sepsis. Why serum RANTES is specifically elevated in survivors within our cohort requires further exploration.

We did not find any other significant differences in peritoneal, pre- or post-operative serum cytokine concentrations between survivors and non-survivors at primary

SCL. In order to elucidate more information regarding cytokine impact on survival, we performed a subgroup analysis of survivors comparing PAC and VAC groups. We hoped to discern if inflammatory cytokines were higher in VAC survivors over PAC survivors. At primary SCL peritoneal cytokines and growth factors IL-6, IL-5, HGF, FGF-b, and VEGF trended to be much higher in the VAC survivors approaching statistical significance. Pre-op serum concentrations of VEGF, FGF-b, IL-1b, IL-5, IL-4, IL-7 and post-operative serum concentrations of VEGF and IL-7 were all significantly elevated in VAC non-survivors at the second SCL compared to VAC survivors.

It is possible that VAC therapy is able to reduce mortality by reducing inflammatory cytokines and growth factors within the peritoneal fluid and thereby reducing the ability to distribute systemically. However, VAC is unable to completely minimize the accumulation of inflammatory cytokines and growth factors within the serum, and once they are significantly elevated (within the serum) mortality increases.

Peritoneal concentrations of growth factors such as VEGF and FGF-b promote wound healing in patients treated with VAC (234). Elevated peritoneal concentrations of IL-6, IL-8, VEGF and FGF have been measured in traumatic wounds undergoing VAC treatment suggesting an improved local environment for wound healing. Furthermore, prospective observational studies and randomized trials have shown that the FGF growth factor family improves healing in burns, ulcers, suture wounds and graft wounds (235). These studies support the local benefit of growth factors, however systemic elevations of growth factors can contribute negatively in sepsis.

VEGF plays a dual role in inflammation and sepsis. It is known to contribute to endothelial impairment and vascular leak (236), yet is also needed for endothelial

survival (237). An animal model of sepsis has shown that endostatin can block serum levels of TNF-a, IL-1b, IL-6 and VEGF leading to improved mortality by reducing vascular permeability (238). These findings provide further evidence of a biochemically more severe sepsis with elevated peritoneal concentrations and a survival benefit provided by removal of peritoneal effluent via VAC management. It also provides indirect evidence of serum VEGF contributing to endothelial damage and associated mortality. Despite these findings, VEGF has not been shown to be associated with sepsis induced multi-organ dysfunction in medical ICU patients (239).

At second SCL patients are no longer in the early phase of surgical abdominal sepsis and non-survivors could have potentially have entered a state of immune paralysis that contributes to mortality. Levels of IL-7 were potentially elevated secondary to T lymphocyte depletion. We did not measure CD4 and CD8 T lymphocytes and therefore cannot say for certain if VAC non-survivors were in fact in a state of immunosuppression and exhibited lymphopenia. Despite this, IL-7 has shown to reverse the effects of sepsis induced lymphocyte apoptosis by increasing CD4 and CD8 T lymphocytes (240) thereby boosting lymphocyte proliferation and immunity (241). Regarding IL-7, our results confirm previous reports that IL-7 is indeed elevated in severely septic non-survivors compared to survivors (180). Indirectly, our data provides evidence that VAC non-survivors systemically are in a state of immune paralysis because IL-7 is increased in response to lymphopenia at second SCL.

7 VAC patients underwent a planned re-laparotomy (second SCL) and there was significant elevations of peritoneal MIP-1a, HGF and reduced IL-10 compared to the primary SCL. Elevations of MIP-1a and HGF may indicate that patients undergoing the

first planned re-laparotomy (second SCL) had a peritoneal environment that was recruiting more immune cells for tissue regeneration and inhibiting the deleterious effect of pro-inflammatory cytokines. IL-10 reduction at second SCL may indicate dampening of the pro-inflammatory cytokine storm. TNF-a and MIP-1b were significantly elevated while IL-2R was significantly decreased at the third SCL (second planned re-laparotomy) compared to the primary SCL. Elevations of TNF-a and reduction of IL-2R provides evidence of a secondary pro-inflammatory response in late surgical abdominal sepsis is possible and it also provides evidence that the VAC survivors at the third SCL do not enter a state of non-reversible immune paralysis.

When peritoneal cytokine measurements were drawn at primary SCL, VAC treatment had not been initiated. The elevated serum concentrations of anti-inflammatory cytokines and growth factors in VAC non-survivors indicate that in certain patients a severe systemic immune paralysis can occur, resulting in mortality. At which cytokine concentration threshold or which time point this occurs at is unknown. VAC patients are able to overcome and survive significantly elevated peritoneal IL-6, IL-17, and IL-5 at primary SCL. Additionally, VAC survivors trended to have significantly higher peritoneal concentrations of IL-6, IL-5, HGF and VEGF compared to PAC survivors. The VAC non-survivors likely surpassed a serum cytokine concentration threshold that was not survivable despite VAC removal of peritoneal fluid. The pro-inflammatory response in these patients induced a strong anti-inflammatory response with no significant elevations in pro-inflammatory cytokines. This likely led to a state of immune paralysis that was non-recoverable compared to VAC patients that had survived until the third SCL.

During a planned re-laparotomy if there is a question regarding proper source control and/or appropriate time to close the fascia, a cytokine analysis may be beneficial to the surgeon. If combinations of IL-6, IL-17, IL-5 and HGF are elevated at primary SCL or remain elevated at re-look laparotomies, initiation or continuation of VAC management is likely required.

CHAPTER 5 Conclusions

We have identified that both pro and anti-inflammatory cytokine concentrations are elevated in serum and peritoneal fluid at primary SCL. This is in accordance with the concept of a mixed anti-inflammatory response syndrome.

Significantly elevated peritoneal concentrations of IL-6, IL-17, IL-5 and HGF at primary SCL within the VAC group compared to PAC provides molecular evidence that the VAC group biochemically had a more severe degree of abdominal sepsis. VAC survivors have non-significantly elevated peritoneal cytokine and growth factor concentrations compared to PAC survivors supporting the association of VAC as a disease modifying treatment. The removal of peritoneal fluid likely helped reduce the deleterious effects of a pro-inflammatory environment locally and reduce the impact of elevated serum cytokines. We presume that if VAC therapy was not initiated in those with elevated peritoneal cytokine concentrations, the pro-inflammatory process would have continued and potentially would have become much worse.

Compartmentalized abdominal sepsis does occur in severe abdominal sepsis and septic shock. Passage of peritoneal fluid from the abdominal cavity to the systemic circulation is likely impaired due to lymphatic blockage in patients who undergo VAC treatment at primary SCL. This is indirectly evidenced by non-significantly elevated pre-operative serum cytokine concentrations compared to PAC. As damage control surgery is performed, peritoneal cytokines are re-distributed systemically with significant increases in both pro- and anti-inflammatory cytokines in serum of VAC cases post-operatively. The pro and anti- inflammatory process in the abdomen does not significantly drop off after primary source control laparotomy. It is dynamic and evolving with interplay

between pro- and anti-inflammatory relationships. VAC survivors are able to mount a secondary pro-inflammatory immune response within the peritoneal cavity evidenced by elevated levels of TNF-a at third SCL. Contrarily, VAC non-survivors display evidence of systemic immune paralysis with elevations in serum growth factors and anti-inflammatory cytokines, specifically IL-7.

We have described new information regarding the relationship of peritoneal IL-6, IL-17, IL-5 and HGF in surgical abdominal sepsis patients. We confirmed our original hypothesis that VAC patients would have a more severe abdominal sepsis expressed as an increase in pro-inflammatory peritoneal cytokine concentrations. However, we did not anticipate the relationships between IL-6, IL-17, IL-5 and HGF within the peritoneum. Combinations of these cytokines have not been previously documented in human abdominal sepsis. They demonstrate potential to act as a group of biomarkers that can indicate severity of abdominal sepsis. Additionally, they may help guide surgical management by suggesting which patients may benefit with VAC therapy. According to our data, elevation of RANTES in pre-operative serum is protective in abdominal sepsis and this new finding requires further validation.

The strength of our study is within our methodology. We were able to identify the impact of damage control surgery, provide biochemical evidence for which patients received VAC treatment, and characterize a longitudinal inflammatory process during re-look laparotomies. A larger cohort of severe abdominal sepsis/septic shock patients requiring urgent source control surgery managed via VAC or PAC is needed to confirm our findings. Our study provides a template to further characterize the inflammatory response in critically ill patients who undergo damage control surgery for abdominal

sepsis requiring open abdomen management with NPWT**.** As the surgical management of this patient population evolves the pathophysiology will change. We must continue to learn how our management affects the immune response so that we can continue to improve patient outcomes.

Limitations

This was a prospective observational study limited by a small population size. Prior to the start of our study we anticipated recruiting approximately 30-40 patients. The beginning of the study proved to be more difficult than anticipated with several factors limiting our recruitment process.

The patient population was critically ill and required at minimum and an urgent SCL was required. Identifying, consenting, and obtaining pre-operative samples proved to be initially challenging with a short time frame for one individual. Reviewing consent with patient family members in a timely and efficient manner improved as the study progressed.

A potential limitation of our study includes surgeon selection bias. Patients were not randomized to receive PAC or VAC management. Surgeon preference, experience or other factors may have influenced outcome. However, within the literature it is accepted that damage control surgery with open abdomen management is reserved for "sicker" patients. The goal of our study was to identify if patients who received VAC had a more severe degree of abdominal sepsis (based on increased cytokine concentrations) compared to those patients who received PAC. Therefore, the effect of potential selection bias was reduced.

Another limitation was the maximum observed concentration of IL-6. The majority of peritoneal concentrations for IL-6 in the VAC group exceed the maximum detectable range. This was reflected in our results, in which statistical comparisons of IL-6 between groups was limited due to the maximum detected range of IL-6.

Cytokine profiles can change depending on the timing of serum or peritoneal fluid collection, patient's age, co-morbidities, malignancy, source of sepsis and potential genetic susceptibilities. However, we addressed this issue objectively by measuring severity of disease with the APACHE-IV PMR score. The APACHE-IV takes into account age, physiological status, medical co-morbidities and other variables. It is the most up to date tool to measure and stratify disease severity that is currently available.

TABLES

Table 1. PAC and VAC prospective case series demographics					
Characteristics	PAC (4 cases)	VAC total (8 cases)		VAC w/o nPanc (4 cases)	VAC w/ nPanc (4 cases)
Age (mean)	60.8	56.5		59	54
Sex (F)	2	2		1	1
Pre-SCL1 Septic shock	3	8		4	4
Deceased	1	3		2	1
GI perforations	3	1		1	-
Anastomotic leaks	0	1		1	-
C. difficile	1	1		1	-
Bowel ischemia	-	1		1	-
Nec Panc	0	4		-	4
Malignancy	1	3		2	1
APACHE-IV score	84.5	89.5		-	-
APACHE-IV PMR	47.6%	43.3%		-	-

Table 2. Pre-operative serum cytokine concentrations at primary source control laparotomy					
Pg/ml	VAC 8 cases Median conc	VAC Interquartile range,	PAC 4 cases Median conc	PAC Interquartile range	P value Mann whitney U
TNF-a	2.5	0.69	10.7	186	-
IL-1b	7.2	8.5	12.5	241.6	-
IL-2	0.98	1.8	0.98	1.2	-
IL-6	506	615	377.2	12932	-
IL-8	174	517	378	7323	-
IL-12	195	116	186	286	-
IL-17	2.3	1.8	2.3	3	-
IFN-a	114	127	147.9	288.3	-
IFN-y	2.3	1.8	3.6	25.5	-
MIG	60	145	32	39	-
IP-10	45.4	103	68.2	3729	-
MIP-1a	35.2	29	39.5	264	-
MIP-1b	82	149	254	3518	-
MCP-1	724	1054	1256	40862	-
RANTES	3656	4731	2854	7241	-
IL-1Ra	604	431	1115	9589	-
IL-2R	842	1963	904	895	-
IL-4	12.5	7.3	11.7	7.7	-
IL-5	2.2	3.5	0.06	7.7	-
IL-7	18.5	137	44.7	149	-
IL-10	62.8	56	119.3	2087.6	-
IL-13	2.9	9.3	1.0	1	-
IL-15	127	224	171	1110	-
G-CSF	67	176	622	5537.8	-
GM-CSF	0.145	2.1	0.04	1.7	-
HGF	2868	3740	963.6	1325.1	-
FGF-b	23	21	18.7	35.7	-
VEGF	5.9	6.2	5.3	81.5	-
EGF	7.3	21	14.2	22.9	-
Eotaxin	36	51.9	92	1122	-

Table 3. Post-operative serum cytokine concentrations at primary source control laparotomy					
Cytokines	VAC 8 cases Median conc pg/ml	VAC Interquartile range, range	PAC 4 cases Median conc pg/ml	PAC Interquartile range, range	P value Mann whitney U
TNF-a	3.2	0.68	3.2	97	-
IL-1b	9.1	5.4	11	18.2	-
IL-2	1.5	2.2	0.82	1.7	-
IL-6	622	800	280	12909	-
IL-8	366	1215	433	1215	-
IL-12	215	63	166	152	-
IL-17	2.6	2.2	2.3	2.5	-
IFN-a	111	81	132.3	55.3	-
IFN-y	3.6	2.6	4.3	2.9	-
MIG	62	128	47	36	-
IP-10	44	83	54	111	-
MIP-1a	39	44	38	14.4	-
MIP-1b	110	129	136	285	-
MCP-1	1046	549	737	2754	-
RANTES	5956	3845	4187	5549	-
IL-1Ra	513	1235	793	7077	-
IL-2R	700	1450	599	454	-
IL-4	12.5	2.9	11.7	8	-
IL-5	2.2	3.8	1.4	5.6	-
IL-7	42	43	59.6	104	-
IL-10	121	74	116.4	391.0	-
IL-13	7.4	7.9	0.68	4.8	-
IL-15	134	170	145	201	-
G-CSF	98	129	376	3311.7	-
GM-CSF	0.17	2.9	0.25	3.4	-
HGF	2716	4210	966.9	2847.1	-
FGF-b	26.7	17.4	16.8	6.7	-
VEGF	7.4	6.2	5.3	17.8	-
EGF	23	16	10.5	17.3	-
Eotaxin	45	30	57	58	-

Table 4. Peritoneal cytokine concentrations at primary source control laparotomy					
Cytokines	VAC 8 cases Median conc pg/ml	VAC Interquartile range, range	PAC 4 cases Median conc pg/ml	PAC Interquartile range, range	P value Mann whitney U
TNF-a	2.8	15.3	1.8	424	-
IL-1b	10.3	84	8.7	14.5	-
IL-2	1.5	2.6	0.7	0.8	-
IL-6	17176	n/a	10434	15550	0.037
IL-8	11201	26605	483	4134	-
IL-12	264	252	214	253	-
IL-17	6.3	6.4	1.3	2.5	0.04
IFN-a	256	159	169	181.9	-
IFN-y	16.2	11.7	3.6	6.5	0.05
MIG	20	76	20.8	44	-
IP-10	123	449	122	143	-
MIP-1a	65	104	43	93	-
MIP-1b	129	1826	189	887	-
MCP-1	4715	16994	2357	25458	-
RANTES	330	1081	24	10729	-
IL-1Ra	1453	8223	554	5114	-
IL-2R	964	2095	499	562	-
IL-4	8	8.8	5.8	13	-
IL-5	8.3	7.6	4.3	5.3	0.026
IL-7	103	57	74	64	-
IL-10	242	739	151.1	1199.6	-
IL-13	9.9	8	5.1	4.9	-
IL-15	349	485	253	195	-
G-CSF	1003	6328	2910	5640	-
GM-CSF	1.5	1.5	0.7	3.4	-
HGF	3204	7121	618.9	677.7	0.042
FGF-b	59	44	33.2	20.6	-
VEGF	44.5	38	23.8	33.4	-
EGF	11.6	14.9	16.5	35.8	-
Eotaxin	270	678	116	1283	-

Table 5. Serum cytokines pre and post primary source control laparotomy in VAC cohort			
Cytokines	Serum pre-op Median pg/ml	Serum post-op Median pg/ml	Wilcoxon signed ranks P value
RANTES	3656	5956	0.025
IFN-y	2.3	3.6	0.028
IL-10	62.8	121	0.05
MIP-1b	82	110	0.05
EGF	7.3	23	0.05

Table 6. Peritoneal cytokine concentrations at primary and 2nd SCL			
Peritoneal Cytokines	VAC at primary SCL (8 cases) Median pg/ml	VAC at 2nd SCL (7 cases) Median pg/ml	P value (Wilcoxon)
IL-10	242	64	0.028
HGF	3204	7497	0.043
MIP-1a	65	119	0.018

Table 7. Peritoneal cytokine concentrations at primary and tertiary SCL			
Peritoneal cytokines	VAC at primary SCL (8cases) Median pg/ml	VAC at 3nd SCL (5 cases) Median pg/ml	P value (Wilcoxon)
TNF-a	2.8	6.2	0.043
MIP-1b	129	765	0.043
IL-2R	964	469	0.043

Table 8. Effect of necrotizing pancreatitis on peritoneal cytokines at primary source control laparotomy				
Peritoneal Cytokines	PAC Median conc (pg/ml)	VAC w/o nPanc Median conc pg/ml	VAC w/ nPanc Median conc pg/ml	P value (Kruskal-Wallis)
IL-2	0.7	3.3	0.8	0.039
IL-17	1.3	8.3	2.6	0.030
IFN-y	3.6	20.8	9.3	0.019
G-CSF	2910	6203	377	0.042

Table 9. Pre-op serum cytokines at primary SCL in survivors and non-survivors					
Cytokine serum Pre SC1	Survivors (8 cases) Median conc pg/ml	Survivors Interquartile range	Non-Survivors (4 cases) Median conc pg/ml	Non-Survivors Interquartile range	P value (Mann-Whitney U)
TNF-a	2.5	0.7	3.5	183.6	-
IL-1b	9.7	11.0	2.4	76.3	-
IL-2	1.1	1.1	0.5	9.7	-
IL-6	468.8	822.1	470.5	613.5	-
IL-8	212.4	553.8	318	503	-
IL-12	195.1	169.2	140.0	120.8	-
IL-17	2.6	1.8	2.0	232.2	-
IFN-a	119.9	116.9	161.1	228.9	-
IFN-y	3.3	4.1	1.7	1.0	-
MIG	32.3	133.7	60	78.9	-
IP-10	36	200.6	89	71	-
MIP-1a	36.3	27.8	38.4	68.7	-
MIP-1b	89.6	171.5	127.3	346.8	-
MCP-1	461.8	1881.7	1264	1434.4	-
RANTES	4951.6	2777	1065.9	1496	0.042
IL-1Ra	520.1	2866.7	680.8	420.9	-
IL-2r	882.8	1240.0	833.7	1858.3	-
IL-4	13.2	2.5	5.8	19	-
IL-5	0.8	4.2	1.4	3.5	-
IL-7	31.3	136.5	31.8	140.3	-
IL-10	46.8	68.0	101.5	160.8	-
IL-13	5.2	12.2	0.7	1.3	-
IL-15	96.0	258.6	176.2	152	-
G-CSF	69.5	228	154.7	878	-
GM-CSF	0.04	1.7	0.4	2.1	-
HGF	1164.7	2529.0	3800	4579.9	-
FGF-b	21.5	17	20.3	75	-
VEGF	6.0	7.1	4.8	5.7	-
EGF	10.8	21.6	5.3	27.0	-
Eotaxin	36.1	90.9	77.0	89.6	-

Table 10. Serum cytokines post-primary SCL in survivors and non- survivors					
Cytokine serum Pre SC1	Survivors 8 cases Median conc pg/ml	Survivors Interquartile range	Non-Survivors 4 cases Median conc pg/ml	Non-survivors Interquartile range	P value (Mann-Whitney U)
TNF-a	2.8	0.7	3.5	96.9	-
IL-1b	9.5	8.7	8.7	47.9	-
IL-2	1.1	1.6	1.45	7.4	-
IL-6	783.5	1021.5	360.7	344.0	-
IL-8	461.4	446.9	272.3	391.2	-
IL-12	201.0	139.6	1680.9	258.1	-
IL-17	2.3	1.1	2.9	142	-
IFN-a	117.0	69.0	156.0	174.7	-
IFN-y	4.3	2.2	3.0	2.9	-
MIG	47.2	121.2	62	59.3	-
IP-10	41.2	129.7	66	60.1	-
MIP-1a	39.3	14	38.1	44.4	-
MIP-1b	110.4	134	129.7	290	-
MCP-1	1046.3	861.4	1005.8	593.7	-
RANTES	6822.7	3587.1	2847.5	4235.9	-
IL-1Ra	885.1	2676	510.0	370.0	-
IL-2r	643.5	1337.9	560	905.9	-
IL-4	12.5	2.2	13.2	13.1	-
IL-5	2.8	3.8	0.8	3.9	-
IL-7	39.6	43.3	77.9	111.3	-
IL-10	121	121.3	91.4	366.6	-
IL-13	8.1	8.7	1.6	4.2	-
IL-15	90.9	216.7	178.5	112	
G-CSF	100	227.5	162.3	515.1	-
GM-CSF	0.2	3.4	0.45	3.0	-
HGF	1597.8	3301.6	2745.8	4524.1	-
FGF-b	22.6	11.8	31.8	38.0	-
VEGF	8.8	9.1	5.3	3.4	-
EGF	18.9	12.4	22.4	23.7	-
Eotaxin	41.5	28.8	67.9	27.0	-

Table 11. Peritoneal cytokines at primary SCL in survivors and non-survivors					
Cytokine serum Pre SC1	Survivors 8 cases Median conc pg/ml	Survivors Interquartile range	Non-Survivors 4 cases Median conc pg/ml	Non-Survivors Interquartile range	P value (Mann-Whitney U)
TNF-a	2.5	16.2	2.8	423.4	-
IL-1b	10.9	85.8	10.3	10.5	-
IL-2	0.8	2.2	1.1	3.0	-
IL-6	17176	10112.6	17176	17176	-
IL-8	748.8	21777.8	3391.1	22231.5	-
IL-12	256.2	313.7	258.1	142.6	-
IL-17	2.3	6.7	4.2	9.2	-
IFN-a	181.9	206.2	255.9	108.9	-
IFN-y	6.8	14.8	16.2	9.3	-
MIG	13.4	45.1	27.3	69	-
IP-10	84.3	280.0	171.2	351.1	-
MIP-1a	43	101.0	83.3	93.3	-
MIP-1b	188.6	1817.8	129	900	-
MCP-1	2357	8899.3	13728.5	27180.3	-
RANTES	330.4	1420.0	23.9	383	-
IL-1Ra	1078.2	9532.3	1056	1497.5	-
IL-2r	1006.1	2255.8	652.9	315.7	-
IL-4	9.5	14.4	7.3	3.7	-
IL-5	7.2	10.4	6.8	2.5	-
IL-7	86.4	61.9	117	39.5	-
IL-10	203.2	773.1	257.4	1202.0	-
IL-13	6.6	11.9	8.7	2.6	-
IL-15	241.8	368	370.6	295.6	
G-CSF	1295	4620.7	4143.3	6699.4	-
GM-CSF	1.1	3.3	1.6	1.3	-
HGF	1528.8	3084.6	3708.4	7437.2	-
FGF-b	49.2	38.3	36.6	148.5	-
VEGF	37.1	48.4	45.1	27	-
EGF	16.3	34.5	10.1	11.4	-
Eotaxin	152	720.1	272	1261	-

Table 12. Peritoneal cytokine concentrations at primary SCL in PAC and VAC Survivors			
Cytokines	PAC Survivors (3 cases) Median conc pg/ml	VAC Survivors (5 cases) Median conc pg/ml	P value Mann-Whitney U
IL-6	3692	17176	0.051
IL-5	2.8	9.9	0.053
HGF	333	2580	0.053
FGF-b	24.7	61	0.053
VEGF	16.5	45	0.053
IL-13 *pre-operative serum	3.3	13.8	0.050

Table 13. Pre-operative cytokines at secondary SCL in survivors and non-survivors			
Cytokines	VAC survivors Median conc pg/ml	VAC Non-survivors Median conc pg/ml	p-value Mann-Whitney U
VEGF	2.3	5.0	0.034
IL-1B	5.7	10.7	0.032
FGF-b	15.3	25.7	0.034
IL-5	0.06	2.8	0.028
IL-4	11	13.9	0.048
IL-7	32.2	63.8	0.034

Table 14. Post-operative cytokines at secondary SCL in survivors and non-survivors			
Cytokines	VAC survivors Median conc pg/ml	VAC Non-survivors Median conc pg/ml	p-value Mann-Whitney U
VEGF	3.1	5.1	0.034
IL-7	18.5	83.7	0.028

Figures

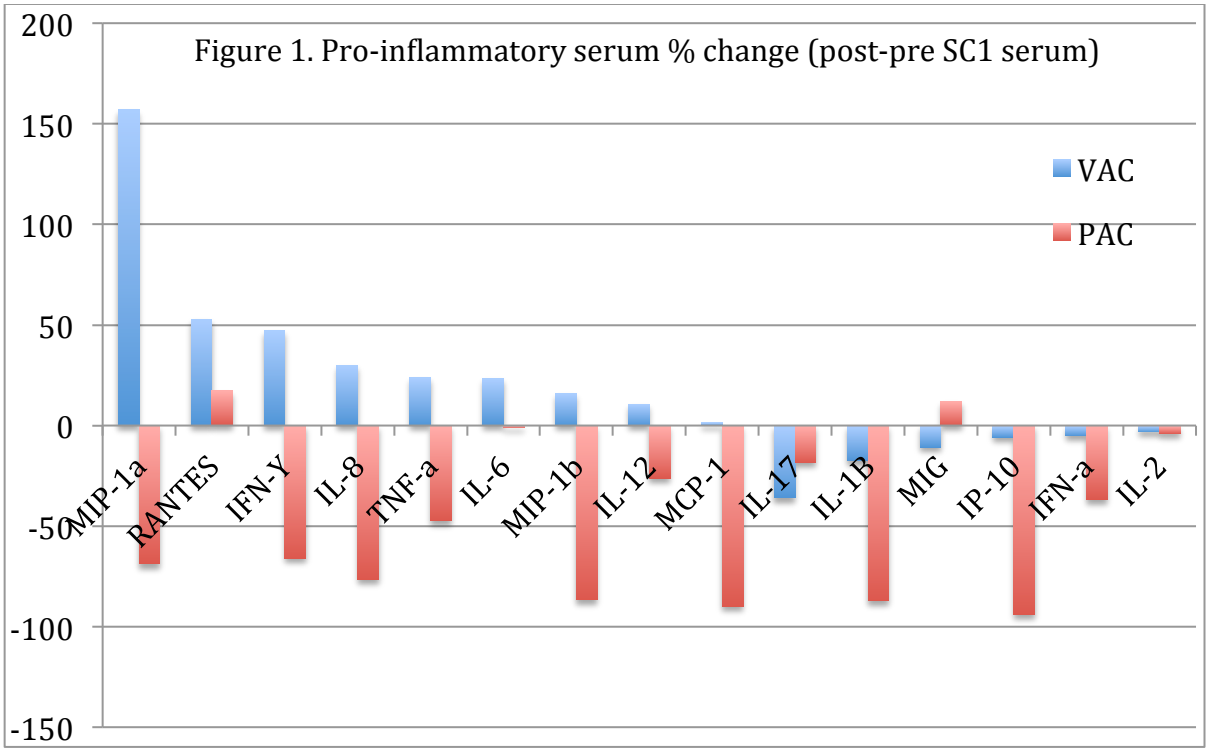
Figure 1. Pro-inflammatory serum % change (post-pre SC1 serum)
200
150
100
50
0
-50
-100
-150
VAC
PAC
MIP-1a
RANTES
IFN-Y
IL-8
TNF-a
IL-6
MIP-1b
IL-12
MCP-1
IL-17
IL-1B
MIG
IP-10
IFN-a
IL-2

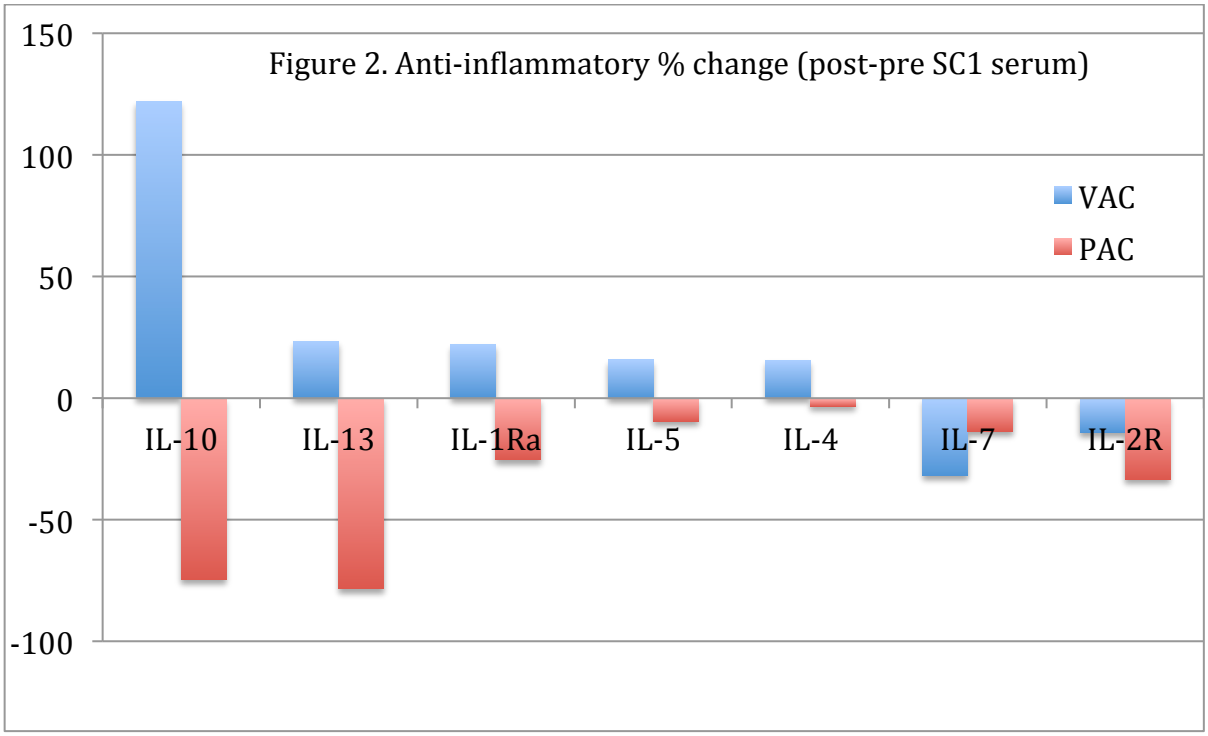
Figure 2. Anti-inflammatory % change (post-pre SC1 serum)
150
100
50
0
-50
-100
VAC
PAC
IL-10
IL-13
IL-1Ra
IL-5
IL-4
IL-7
IL-2R

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